

## Who will exploit academic ideas?

*The wheel has come full circle in Britain, where the government is about to remove a monopoly on public innovations. Universities hope to benefit. They may not.*

IMMEDIATELY after the Second World War, the then British Government was persuaded (chiefly by the late Lord Blackett) that steps should be taken to make fuller use of British inventions. The result was an organization called the National Research and Development Corporation (NRDC), a publicly-supported entrepreneur given a first option on the exploitation of innovations arising from the use of public funds. Last week (see *Nature* 15 September, p.172) Mrs Margaret Thatcher, the Prime Minister, reminded her special seminar on technology and "wealth creation" that too many British innovations were being exploited by other nations — and then announced that NRDC would lose its right of first refusal. The objective now, as in 1947, is to wring more benefit from home-grown inventions. What has changed — or gone wrong? The question is important not merely for British taxpayers but for most other industrial states looking for more prosperity from innovation.

Those most directly affected by the government's decision, NRDC itself and the rump of the old National Enterprise Board with which it is now united in the British Technology Group (BTG), will probably be relieved by Mrs Thatcher's announcement. The threat that something like this was about to happen has been public knowledge for nearly two years. For much of that time, inventors have been puzzled to know what they should do with their bright ideas, a recipe for neglecting them. In practice, last week's decision will be widely, even enthusiastically, welcomed. But there is a danger that people — inventors and the government — will now expect too much. It is far from clear that NRDC's difficulties can be avoided by others.

NRDC has been through several cycles of up and down, but outwardly it has always been a profitable organization. The principle was that the government (at first the Treasury, more recently the Department of Industry) would provide a stock of capital, that NRDC would sift through all the bright ideas within its purview, take out patents on those that looked promising and carry them through to the marketplace by investing (sometimes in partnership with others) in their development. While in principle the public capital (£50 million at its peak) was always totally at risk, NRDC has been lucky always to have had a money-spinner on its books. At the outset the wartime Bailey bridge helped to balance the books. Since 1960, the cephalosporin antibiotics have taken over that role. In its time NRDC has also backed several conspicuous failures — hovercraft, linear motors for train propulsion, fuel cells that were overtaken by events and the "Dracone" (a way of transporting petroleum by sea in a huge sausage pulled by tugs). Such are not, however, the reasons why NRDC has become unpopular. It has been forgiven its errors of commission, but its inclination to say no has made it an army of enemies.

Not all that criticism can be sustained. Like those who write books, inventors often have a keener appreciation of the merits of their works than of their defects. They naturally visit their wrath on those who decline to back financially what they have done. There is no proof that NRDC was ever inconsistent in its judgements. What is clear, however, is that NRDC was often slow to take a bright idea onto its books and that, when it had done so, it would fail to give the originator of the project a good reason for skimping on development costs.

The underlying difficulty, for everybody concerned, is that the

scale of NRDC's operations was always in principle arbitrary — at any stage it could have been investing at twice or half the going rate without knowing which course would in the long run be more profitable. So, in practice, it played safe, and lived within its assured income, hoping never to be accused of gambling unsuccessfully with public funds. NRDC's monopoly right has been in jeopardy at least since the occasion a few years ago when the Medical Research Council successfully argued for a waiver from the government that allowed it to sign a first refusal deal with Celltech, the then new biotechnology company. What the government now intends is that NRDC should stay in business (within BTG) but compete with other entrepreneurs (all private) for patentable inventions to back. British universities and academics are looking forward to being rich. But they are likely to be disappointed.

Even so, the decision to end the monopoly is right. When there were virtually no sources of funds (venture capital is the usual term), one was plainly better than none. In the present circumstances, with merchant banks and *ad hoc* venture funds prowling around universities and other public places, the monopoly is an anachronism. The essence of the relationship between an innovator and his backer is that the backer should share some of the enthusiasm of the inventor, should nevertheless have a capacity to decide quickly (if wrongly) when a project has gone wrong, and should be willing to go on his hands and knees to others for extra capital if (the rule, not the exception) a development project remains promising but costs more than originally foreseen. It is inappropriate that salaried public servants should attempt all these roles using public money. NRDC's record shows that it has not tried to do so vigorously, even if it can take credit for having heightened the interest of academics in innovation and its exploitation.

What will happen next is an open guess. To begin with, the universities will be most directly affected. Corporately, their difficulty will be to choose between activity (seeking out new ideas, even taking out patents) and passivity (letting academics who consider their work to be ripe for exploitation take the initiative. There are strong arguments against too much activity — it is distracting, expensive and there can be no assurance that universities will be more imaginative than NRDC. But passivity implies a return to the period before NRDC, although with the difference that there are more organizations willing to invest in novel projects. Perhaps that development is NRDC's most enduring success. □

## Policy by merger

*Taking scissors and paste to the US bureaucracy is fashionable but could be dangerous.*

THE Reagan Administration has a poor record on the reorganization of the federal bureaucracy. It came to power pledging to abolish the Departments of Education and Energy. Both, for different reasons, continue. Now attention in Congress is being transferred to a new administration proposal — merging the Commerce Department with the White House Office of the US Trade Representative in a new Department of International Trade and Industry. This idea, reflecting as it does the



exaggerated awe with which many people in the United States regard Japan's Ministry of International Trade and Industry, is likely to do rather better. But many rough edges remain to be smoothed out.

One problem is what to do with the various unwanted limbs with which the Commerce Department has become unwillingly encumbered, and which it would like to excise when it moves into its new role. The thorniest of these — the National Bureau of Standards (NBS) — would, under the administration plan, be merged with the National Science Foundation (NSF) on the slender grounds that both agencies deal with science and could therefore form the nub of a powerful new body responsible for enhancing the vitality of science and technology in universities and industry. In fact, the two agencies have precious little in common. NBS, with its origins in weights and measures, operates its own laboratories, concerns itself with measurements and sets national standards. NSF is a grant-giving body, precluded by law from operating its own laboratories, with a record of failure in attempts to stimulate applied research or technology.

The administration argues that joining the two bodies would be synergistic. What is more likely is that they would be mutually enfeebled. NSF, under the leadership of a new director, is already in the throes of a significant internal reorganization designed to strengthen its fundamental objective — the support of good research. Attaching NBS and putting both organizations under a single budget, and under the guidance of the National Science Board, is a recipe for internal friction and uncertainty about the new agency's priorities.

Another orphan cast out from the Commerce Department might be more than eager to leave. The National Oceanic and Atmospheric Administration (NOAA) is likely to fare better as an independent agency, as proposed in the reorganization plan, than it has as an unloved adjunct of Commerce. Conceived in the 1960s as a "wet" version of the National Aeronautics and Space Administration (NASA), NOAA has been at loggerheads with its parent department for much of its life. Congressional supporters of NOAA complain that the Commerce Department is not a strong enough advocate of the agency's needs during the budget-making process. Friction has been increased this year by the department's insistence that NOAA should seek commercial buyers for its meteorological and remote-sensing satellites. A separation between the two agencies is possible even if the overall plan for a new Department of Trade and Industry comes to naught.

Politically, that is the most likely outcome. Enthusiasm for the new Department of International Trade and Industry is confined to the Senate, and even there the idea will encounter the usual opposition from committees which expect the dismemberment of Commerce to curtail their own jurisdictions. It would be a pity, however, if Congress missed the opportunity which the scheme provides for a hard look at the tangle in which the United States has wrapped itself over international trade policy. There are important issues for the United States to resolve. How, for example, can it continue to call for the promotion of high-technology exports while pressing obsessively for the restriction of technology transfers to unfriendly nations? And the inconsistencies of US policy towards Japan are glaring. Reorganizing the federal bureaucracy is seldom an adequate solution to fundamental policy questions, but it can help to sharpen their definition. □

## Aircraft and missiles

*Why not use the Korean aircraft tragedy to build a missile monitoring system?*

HERR Dietrich Genscher, the West German foreign minister, should have expected the quick rebuff from the Soviet Union earned by his attempt to smooth out the way towards an agreement on arms control at Geneva. After his meeting with Mr Andrei Gromyko, Herr Genscher hinted publicly that the Soviet Union was in a mood to drop its demand that the French and

British nuclear forces should be counted in the intermediate-range missile talks, but only on condition that they were counted as strategic weapons under the START negotiations. However, the Soviet Government has now made it plain that nothing of the kind is intended. Furthermore, the Soviet Union has said that its negotiating position will not be influenced by what happened off the island of Sakhalin the previous week. Should that stern edict be allowed to stick?

The most generous explanation of the destruction of the South Korean airliner is that the Soviet eastern air defences were incompetent in tracking aircraft in their vicinity and either inconsistent in telling what kind of aircraft had flown over Soviet territory or callous in deciding to destroy it. Less generous explanations are that the action was deliberate, in which case it was a blunder in a wider sense. It is natural that people in the West should go on repeating statements such as these (but often stronger), but it is also time to decide what practical benefits there may be in a set of circumstances that will otherwise be simply tragic.

The West Pacific air-lane is not the only route on which military installations are sited. Large tracts of North America are in the same condition, as well as Europe (East and West), but these are few prohibitions. By the evidence of the past two weeks, however, in the West Pacific air-lane civil aircraft and their living contents are more likely to be endangered by simple navigational errors. Moreover, for as long as the Soviet Union refuses an inquiry into this tragic affair, there can be no disproof of that assumption.

The circumstances are those in which a person maintains a boundary of a piece of property in such a condition that passers-by are likely to be injured if they miss their path. In most legal systems, the rule that trespassers should not trespass is overridden by the requirement that landowners have no right to devise cruel and inhumane punishments for those who break a civil injunction.

In the West Pacific, this implies that the Soviet Union should be responsible for making sure that future navigation errors do not lead necessarily to disaster. The International Civil Aviation Organization was advocating in Montreal last week that all civil aircraft should carry transponders that would allow unambiguous and speedy recognition by air defences, but that suggestion is too clumsy. Since it is the Soviet Union's fence that has proved dangerous, surely it is for the Soviet Union to take steps to ensure that aircraft straying from their course are warned by radio, not by having bullets fired at them. A ground-based air control station manned by Soviet forces is therefore the obvious solution.

Such a development would have wide implications. In spite of the dusty answer to Mr Genscher, both aides in the Geneva negotiations appear to have arrived with more workable instructions. Mr Yuri Andropov's promise that SS20 missiles removed from West European targets would be destroyed, not moved further back, apparently stands. And the US negotiators will apparently be free to consider compromises without having to refer back on every detail.

What remains entirely undecided is how compliance with a treaty to limit numbers of intermediate missiles would be verified. (The problem would not of course arise if the agreement were that there should be none on either side.) What kind of arrangement could there be?

The simplest way of policing an agreement on European missiles is also probably the best — a sufficient exchange of intelligence data to persuade each party that its missile dispositions are known to the other. The weakness in such proposals is that they make it possible for one to spot defects in the other's intelligence. But that might not apply if the data were collected and assessed by some neutral third party. A party recognizing gaps in the data applying to himself would also find similar gaps in the other fellow's. So why not use the need for a strictly non-military monitoring post on Sakhalin to demonstrate to all concerned that the independent collection and use of data is feasible and often valuable? □



## French budget

# Spending is slashed but not for science

"THE War Budget" screamed the headline from the French newspaper *Libération* last week, in response to the announcement of one of the most severe French budgets since the Second World War. Government spending in 1984 is to increase 6.3 per cent (about the same as inflation) compared with projected economic growth of 1 per cent. Given a self-imposed limit of a 3 per cent budgetary deficit, the difference means FF 60,000 million (£5,000 million) of new taxes. The results: Prime Minister Pierre Mauroy's popularity in opinion polls has plunged to 25 per cent; and President Mitterrand made an unprecedented 1½-hour appearance on television last Thursday to defend his government's policy. Not the circumstances, one might think, in which an expansionist scientific policy might flourish. But it has, and French thinking now aims at extending this policy to the whole of Europe.

While most government departments are facing constant or reducing budgets in real

circumstances". This is strong praise, from a man who resigned over what he considered to be the government's excessively cautious approach to spending. Research had become rather "a fashion" in France, he said in an interview with *Nature*, "and while I don't believe in fashions this is a good one . . . I'm pleased to think I had something to do with launching it."

Research, development and innovation have become this government's flag of hope. "We might become Greece to Europe's Rome" one ministry official said last week. And both the President and Prime Minister, on separate occasions, emphasized their reliance on a technological new dawn. President Mitterrand, in a ceremony breaking ground for the new accelerator LEP, at the European Organization for Nuclear Research (CERN) near Geneva, emphasized the European connection: Europe must not lose its place in the "third industrial revolution", he said. To keep its place, Europe should follow France, he implied. To this end, France would launch "new initiatives" on research when the presidency of the Council of the EEC next reverts to French hands. (One of these, for example, will concern a real improvement of mobility of researchers in Europe.)

The feeling in Paris is clearly this: France, with its 50 million people, is too small both economically and scientifically

to succeed alone in the brave new world of the 1990s and the next century. For even France to succeed, Europe must join scientific hands. A recent agreement between British, French and West German computer companies to set up a joint research centre in Munich is widely seen in Paris as a good sign: the cooperation should not all be through Brussels, but by a strong network of two-, three- and multi-way agreements — and Sweden, Switzerland and Austria (outside the EEC) should join in too. Thus, shortly, minister Laurent Fabius — a passionately convinced European — plans to visit certain European capitals to drum up support for an extension of such plans. In basic research, Pierre Papon — the director-general of the principal French research council, the CNRS — is also planning a European tour, again aimed at a real increase in cooperative effort. Papon says he is "absolutely certain" that the future of Europe depends on the success of such cooperation.

But, as the Prime Minister Pierre Mauroy said at Orsay (the scientific centre south of Paris) last Monday, it all depends on researchers becoming more involved with industry, and on industry taking account of science. Big industry in France increased its research spending last year, but only a little (about 5 per cent, according to some estimates); and small-to-medium industry has stood still. "State and industry must collaborate to devote a greater part of the national wealth to research and development" said Mauroy. He also urged that researchers be encouraged to create their own businesses — a close echo of the policy of Mauroy's opposite number across the Channel.

Robert Walgate

IMAGE  
UNAVAILABLE  
FOR COPYRIGHT  
REASONS

terms, Laurent Fabius's ministry of industry and research will benefit from a 15.5 per cent increase in monetary terms. Much of the cash will go to support investment in the massive nationalized industry sector, but basic research will still see a 12.5 per cent increase — or 6 to 7 per cent in real terms. The French franc may have lost a lot of ground against the dollar last year, but these figures mean that French science will nevertheless be defended in 1984, and that the political will to support it remains very strong.

Even Jean-Pierre Chevènement, former minister of research and industry and political architect of present French science policy, said last week that he thought the science budget was "good, in the

## CERN breaks new ground

THE French and Swiss Presidents were doing spadework at the European Organization for Nuclear Research (CERN) last week — breaking ground for CERN's next accelerator, LEP, which will straddle the French-Swiss border. CERN, said the French President, had regained for Europe "a dominant place in this domain of physics and high energies" and perfectly illustrated the kind of political cooperation that was now needed in Europe in all sciences. Beams all round.

In the first version, LEP will collide electrons and positrons at sufficient energy to make copious numbers of  $Z^0$  particles, the neutral partners of the triplet ( $Z^0$ ,  $W^+$  and  $W^-$ ) whose exchange among particles results in the weak force. A later version of LEP could enable it to reach the  $W$ s.

However, the reader might say, has CERN not already discovered these particles? Indeed it has, but only in small numbers. LEP is capable of making thousands a day, and such statistics will allow a much more detailed analysis of the

particles — and so provide a severe test of the Weinberg-Salam unified electro-weak model that predicted their existence and properties. LEP will also be able to work at other energies and on other particles — such as the expected top quark.

Even so, given the £300 million cost of LEP, some heart-searching is setting in. The  $p\bar{p}$  collider that found the  $Z^0$  and  $W$ s has been much more effective than expected — and hot competition is coming from California, where the Stanford Linear Accelerator Center (SLAC) is staging a cut-price (£75 million) attack on LEP physics. Dr Burton Richter, Nobellist master-mind of this SLAC "single pass collider" (SPC) said on Monday that his SPC could still be ready by October 1986, compared with LEP's 1988. SPC may take longer to run in than LEP, because of its new design, it may produce only one-quarter the number of  $Z^0$ s, and its detectors will be old, but it does threaten to cream the best of LEP physics before LEP even turns on.

Robert Walgate







## AIDS research

## Is there really a virus?

## Brussels

MINUTE freckles on a photograph of human lymphocytes taken by two Brussels-based medical researchers could, they claim, be images of a virus responsible for acquired immune deficiency syndrome (AIDS). The lymphocytes were isolated from the blood of a Zairean patient under observation at the Brussels University St Pierre Hospital.

The findings of Dr Walter Feremans and Dr Nathan Clumeck of the Electron Microscope Department of Université Libre de Bruxelles (ULB) and four colleagues (*Lancet* ii, 52-53; 1983) were reprinted in Belgium's weekly *La Revue de Medecin*



Virus-like inclusions (V) in a lymphocyte (from *Lancet* ii, 52; 1983).

and caught the eye of a Brussels reporter of the French news agency AFP. Her somewhat sensationalist article stirred up strong chauvinistic feelings in the Belgian press and started what Dr Feremans called "a highly undesirable tidal wave of often inaccurate reports".

In the *Lancet* letter the authors signal the existence of the small spots in lymphocytes of all seven AIDS victims being treated at the St Pierre Hospital. They go on to speculate that the specks could be a virus and could possibly have something to do with AIDS.

The researchers suspected the spots were a virus, but at that stage did not know whether they caused the illness or if the virus had made its way into the cells as a result of the deficiency in the patients' immune systems. "There were indications but we had to be cautious," Dr Feremans said. But research has progressed since the *Lancet* letter, and recent experiments seem to confirm a link between the virus and AIDS.

Understandably, Clumeck and Feremans, who are preparing another article for the *Lancet*, are reluctant to expand on the results of their experiments, but the few indications Feremans is willing to give are intriguing. First, there is increasing evidence that the grey spots on the picture are indeed a virus. "Markings and tests with colloidal gold point at a virus. The virus doesn't seem to be of the human T-cell leukaemia (HTLV) kind," said a cautious Feremans. But what could be a

major breakthrough came up when the team performed blood tests on the wife of one of the seven AIDS patients at St Pierre Hospital. The tests suggest that the woman is suffering from AIDS at a very early, asymptomatic stage. And the electron micrographs again reveal the same grey spots. "We know that after the press campaign, the international scientific community had its heavy artillery in position to shoot us to pieces if our discovery proved to be wrong," said Feremans, "but this does give us hope."

The idea of examining the (black) woman's lymphocytes came up when the team found out the married couple had lost a newborn child only months after its birth in Africa. The child reportedly died of multiple infections. "We identified the woman as an AIDS patient. But the illness is in its earliest stage. The woman's natural

defences are still functioning properly, and in any case would have been sufficient to eliminate or to keep out undesirable viruses," Feremans said.

The next step will be to concentrate on isolating the virus. "The only thing we have done so far is photographing what might be the AIDS virus," he said. The Brussels branch of the Institut Pasteur which prepares the lymphocytes for the experiments is also attempting to isolate the virus.

The massive publicity surrounding Feremans' and Clumeck's experiments, and the chauvinism of the press have highlighted the sorry state of public medical research funding in Belgium. More than 40 cases of AIDS have been identified in the country and twelve deaths have been recorded so far, making Belgium one of Europe's hardest-hit countries. But no extra funds have been made available and coordination between the various Belgian institutes is restricted to the exchange of information because money is scarce.

Geert Linnebank

## Australia

## CSIRO in tangle with government

## Canberra

MEMBERS of the executive of Australia's Commonwealth Science and Industrial Research Organization (CSIRO) are still smarting as a result of a recent directive from the government minister responsible for the organization. The directive, which was sent during pre-budget discussions with the CSIRO executive when the cut in the organization's 1983-84 budget was mooted (see *Nature* 15 September, p. 174), makes bland reading: "I, Barry Owen Jones. . . direct the executive of CSIRO to devote the same proportion of the organization's 1983-84 appropriation to research as was allocated in 1982-83."

The directive has caused a stir because it breaks the traditional relationship between ministers and CSIRO in which CSIRO exercised autonomous control over the administration of its funds. The implication is that Mr Jones fears that research might suffer disproportionately as a result of the cut-backs.

The science and industry research act of 1949 establishing CSIRO, gave the relevant minister wide powers over the organization, which were subsequently curtailed by an amendment to the act in 1978 after an independent inquiry into CSIRO. This restricted ministerial direction to specific policy matters as opposed to administrative arrangements. Even so, as Dr Paul Wild, chairman of the CSIRO executive, wrote in the preface of its last annual report, "I am glad to say that no minister responsible for CSIRO has found it necessary to exercise this power of direction".

The CSIRO executive seems to be at a loss to explain Mr Jones' directive, maintaining that, in any case, it is the

organization's policy to allocate the same proportion of funds to research from year to year. They sought clarification from Mr Jones, who replied that CSIRO should cut back overheads and support services, not research.

The lack of rapport between the minister and the organization may have begun at the change of government earlier this year when CSIRO's executive tried unsuccessfully to wrest the organization out of the science and technology portfolio and make it responsible to the prime minister, although the move was essentially a bid for greater autonomy. Ministerial suspicion of the organization has subsequently hardened over the issue of the Australian National Animal Health Laboratory (see *Nature* 303, 190 and 403), when Mr Jones sought submissions from private individuals and the bureaucracy without discussion with CSIRO. The banning of foot and mouth virus for 5 years was a blow to CSIRO which now has the unenviable task of manning the laboratory without the specific funding for 85 staff positions they requested as a separate appropriation. Furthermore, the government will be considering other recommendations of the report by the Australian Science and Technology Council which, if adopted, will make importation of other viral pathogens subject to consultations with all interested organizations. The net result would be to enmesh the importing of pathogens, some of which are already in Australian laboratories, within a bureaucratic tangle.

Mr Jones would like to see CSIRO expend more of its efforts on manufacturing research, particularly in the fostering of high-technology industries.

Vimala Sarma



## UK research councils

## Where the cuts may come

A scrutiny of selected research council support services ordered last year by Sir Keith Joseph, Secretary of State for Education and Science, has identified potential savings of £3.3 million a year which could be achieved through purchasing policy and management changes. A further one-off saving of £5.3 million could, according to the report, result from recommendations to the Agricultural Research Council (ARC) and the Science and Engineering Research Council (SERC) to dispose of some of their assets.

Individual reports on the four research councils scrutinized have not been made public and neither have the councils' responses, but the summary report of the

central team appointed by Sir Keith to carry out the scrutiny was published this week. As well as ARC and SERC, the National Environmental Research Council (NERC) and the Medical Research Council (MRC) were examined. These four councils had total budgets of £540 million in 1982-83 and employed 17,000 staff. Sir Keith has welcomed the scrutiny's recommendations, and has written to heads of research councils that "... the scope for greater efficiency and savings ... will be realised only if there is vigorous follow-up action at all levels". The councils have been asked to submit "action documents" on implementing the recommendations by the end of November, and progress will be monitored by the government's efficiency unit.

The most controversial recommendation is that SERC should sell off Herstmonceux Castle in Sussex, which forms part of the Royal Greenwich Observatory (see *Nature* 7 July, p3). Together with houses maintained for staff by SERC and ARC, the sale could, according to the report, raise £4.2 million immediately and save £1.05 million a year. To facilitate disposal of assets identified as "surplus to requirement", the review recommends that a proportion of the proceeds arising from sale should be retained by the research council concerned, rather than, as under present arrangements, be returned to the Treasury. The other savings, which mostly affect non-scientific staff, could be achieved by greater cooperation in pur-

chasing policy (ARC and MRC), improved costing of workshop services and rationalization of library provision (NERC and MRC) and of stores (everyone). Purchasing changes alone could save £1.2 million a year.

The report says there is a need for a general strengthening of procedures for staff inspection, management services studies and internal audits. It is also asserted that too much of the cost of support services has "been hidden or treated as an overhead", and that improvements in costing will ensure that those actually conducting research will bear the responsibility for ensuring value for money. It is recommended that costs be charged to users at programme or project level; one of the examining officers found that amongst users there was "a lack of awareness, a lack of interest, or indeed a feeling of helplessness, about the cost of support services".

Another major theme which emerges from the review is that there is scope for improvement in coordination between different units supported by the councils, both in purchasing policy and in services provided. The scrutiny team also wants heads of research councils to prepare proposals to assess "the value for money obtained from research projects" within their external project reviews.

Although the published report does not indicate how the councils responded to their respective reports, it is known that some of the recommendations relating to the SERC's assets have been contested within the council, on the grounds that they have scientific ramifications outside the scope of the Rayner unit's remit.

Tim Beardsley

## Hopes for Insat-1B

### New Delhi

SATELLITE-based domestic telecommunications, television and meteorological services in India should benefit from the successful launch of Insat-1B in India from Kennedy Space Center in Florida on 31 August. India's space programme had received a severe jolt last September when Insat-1A ran out of its onboard fuel. This prevented the national hook-up on television stations, needed for the coming Asian games, but the ground facilities created at a cost of £200 million were made use of by the hiring of two transponders on Intelsat and one transponder on a Soviet satellite. Insat-1B, stationed at 74 degrees east longitude in the geostationary orbit, will now be able to take over these functions.

One advantage of Insat-1B is that it will reduce the congestion in telecommunication services between the major cities of India. There will be 12 transponders, each with 600 working channels for telecommunications. In addition, two transponders will provide television and other meteorological services. Insat-1C, to be built with the help of Ford Aerospace and Communication Corporation, will replace the satellite abandoned last year.

"Insat-1C would represent a transitional stage between foreign-made Insat-1B and Indian-built Insat-2 test spacecraft which would be available by the end of the decade", says the Indian Space Research Organization (ISRO).

Meanwhile, ISRO says that India's first experimental geostationary communications satellite Apple has achieved all its goals despite a jammed solar panel. Its life is expected to end this month.

Apple project director R.M. Vasagam says the experience gained in launching and using the satellite for technical experiments will help Indian scientists to build the second generation satellites. Sunil Saraf

## UK biotechnology

## Industry joins the universities

THE Institute for Biotechnological Studies, a non-profit-making company established earlier this year by three academic scientists, has launched a £1.4 million research programme in biotechnology funded jointly by industrial companies and the Department of Trade and Industry. The programme, which will last for five years, will employ model systems to investigate generic problems associated with the long-term use of immobilized cells in biosynthesis.

So far four companies have joined the scheme (Shell Research Ltd, Glaxo Group Research Ltd, May and Baker Ltd and Unilever PLC) and the programme may be extended to include up to three more. Industry's contribution will be matched by the government. The institute arose from collaborative teaching and research in biotechnology-related disciplines at the Polytechnic of Central London, University College London and the University of Kent. Last year a report by the institute's directors, Professors Alan Bull, Geoffrey Holt and Malcolm Lilly, prepared for the

Organization for Economic Cooperation and Development, highlighted long-term use of biocatalysts as an area deserving urgent research, and the Department of Trade and Industry indicated its willingness to contribute provided that industry shared the cost. According to Professor Lilly, negotiations were made much easier for industrial companies by having the institute to unite the expertise of three academic departments.

Although the companies involved have different interests, the problems to be investigated are of sufficient general importance to justify the collaboration. Besides having access to research results at a fraction of normal cost, the participating companies will have access to specialist training courses provided by the institute. As the institute's primary aim is educational (it offers advice and will undertake contract research in a wide variety of areas in biotechnology), many of the research findings will be published, although the sponsors will retain a right of veto.

Tim Beardsley



## Soviet Far East

## Not quite a closed area

THE Soviet Far East is not quite the military enclave, bristling with defence high-technology and totally off-limits to foreigners, suggested by reports of the tragedy of the Korean airliner KAL-007 three weeks ago. A letter to *The Times* of London from Professor D.J. Crisp, who reported a brush with the security authorities while examining shore-life with a magnifying glass on the coast near Nakhodka, shows that, at least occasionally, foreigners are allowed into the area. In fact, the Far East (which in Soviet terms stretches from the Bering Straits to Vladivostok), while highly sensitive, is not entirely "closed".

Joint ventures with Japan for exploring and exploiting the Sakhalin offshore oil-fields do exist and, after long years of negotiation, there is a cooperation agreement for fishing the Sea of Okhotsk. There are, moreover, occasional international scientific conferences in the region, on topics such as salmon breeding.

Science in the Soviet Far East has always been orientated towards regional development. The area has valuable fossil fuel, minerals (including precious metals) and marine resources, and the need to coordinate research has been apparent since the late 1960s. In 1970, existing institutes and stations of the Soviet Academy of Sciences in Vladivostok, Magadan, Petropavlovsk-Kamchatskii, Khabarovsk-na-Amure and Yuzhno-Sakhalinsk were amalgamated into the academy's Far Eastern Science Centre, with special responsibility for training specialists and coordinating research.

This programme, for the most part, has been successful. Criticisms in the mid-1970s that its research programmes were fragmented and irrelevant seem mostly to have been due to internal academy politics. The centre concentrates on fish-breeding and marine biology, vulcanology (including the use of geothermal waters for district heating and greenhouses), the agricultural needs of the area (including the newly developed rice paddies), conservation (including regular censuses of the Ussuri tigers and the establishment of new beaver colonies on Kamchatka) and geology.

The centre operates several research vessels, including its flagship *Kallisto*, the *Professor Bogorov*, the *Morskoi Geofizik* and the submersible *Vulkanolog* for examining under-water volcanos. Its latest acquisition, the *Akademik Aleksandr Vinogradov*, arrived in Vladivostok last June from Riga, after a maiden voyage monitoring the ocean atmosphere and river-ocean interfaces, performing geological investigations of the Red Sea depression and participating in a joint Soviet-Vietnamese survey of the Mekong Delta.

Several research projects are of more than local significance. Apart from ob-

viously "international" work done by the Yuzhno-Sakhalinsk satellite monitoring post, the Sakhalin weather centre supplies data to the world meteorological network. And the Kamchatka Vulcanology Institute has become the focus of union-wide studies of possible precursors of nucleic and amino acids in the ejecta of volcanos. Botanical surveys of the Kuriles have identified a number of medicinal plants with ginseng-type properties, in addition to *Eleutherococcus*, a relative of ginseng and a well-known product of the region, regularly used by Soviet cosmonauts.

Fisheries research, carried out jointly by the academy's Institute of Marine Biology and TINRO, the Pacific Fisheries and Oceanographic Institute, has made possible the acclimatization of Siberian salmon in the Baltic and Kamchatka crabs on the Soviet Arctic coast.

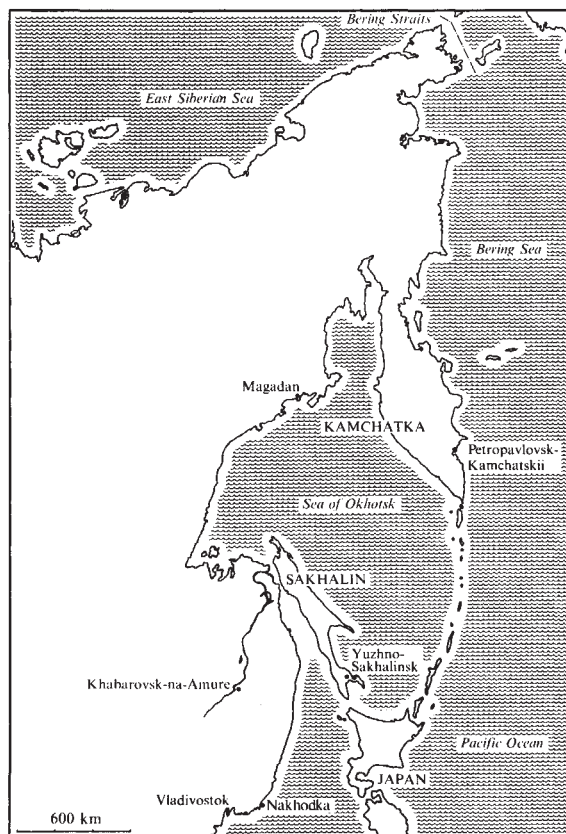
Not all research in the area, however, is entirely successful. The Far East, like the whole of the Soviet Union, suffers from delays and difficulties in implementing research results. In the mid-1970s, planners in the Khabarovsk *krai*, who had been equipped with computers and data-processing aids to planning, were found to be making little or no use of them. TINRO and the Far Eastern Science Centre have this year done considerable work on the rational planning of fishing in the area, have restored the herring shoals of the Sea of Okhotsk and recently launched an integrated salmon breeding and processing pro-

ject that, according to Mikhail Spichak, deputy head of research of the Soviet Ministry of Fisheries, will virtually turn the whole of the Far East into one gigantic fish-farm. One new project, however, the encouragement of the fishing of issuri (Japanese sardine) in the Sea of Okhotsk, although based on the latest ichthyological and ecological research, has been let down by industry, which has failed to produce the necessary processing plants, so that the majority of the catch goes to waste. On the other hand, Lev Bocharov, head of the Applied Mathematics Laboratory of TINRO, admitted recently that the work of his laboratory, which models and forecasts the movement of fish shoals, at present produces only "greater knowledge and scientific calculations" rather than "actual results", which suggests that it is sometimes the scientists who lag behind the demands of production.

In agriculture, although much work has been done on selection and breeding of new strains suited to the climate and poor soil conditions, annual production figures show regular shortfalls. This is particularly serious in the case of soya beans: ten years ago, the Far East produced more than 95 per cent of the total Soviet output. In spite of several multidisciplinary conferences to discuss possible improvements, it has become increasingly apparent that the soil of the area is too poor to meet the planners' targets. Recently, therefore, the emphasis has changed. Instead of producing more beans, the agronomists in the area are acting as advisers to other areas of the Soviet Union where soya cultivation is being introduced.

Vera Rich

*The Sea of Okhotsk — scene of the KAL-007 tragedy — contains important strategic installations, and non-allied foreigners are not encouraged to "paddle about in it", Pravda editor Viktor Afanas'ev said in London this week. But it is also a major growth area for Soviet non-military science.*





## Is CO<sub>2</sub> euthanasia humane?

SIR — David Freed (*Nature* 11 August, p.482) casts doubts on the humaneness of carbon dioxide for the killing of laboratory animals. I believe that Dr Freed's doubts are, to a large extent, correct as long as one is considering relatively large animals.

In mammals larger than guinea pigs there seems to be a delay in the onset of unconsciousness, and the animals appear distressed (restlessness, deep respiration, salivation, pawing at noses) before they collapse. This distress is believed to result mainly from irritation of the lining of the nasal passages by CO<sub>2</sub> going into solution and forming H<sub>2</sub>CO<sub>3</sub>. In smaller creatures, such as rat and mouse, the onset of collapse is quick and it seems the animal is unconscious before local irritation of the mucus membranes sets in. It seems that, in small animals, CO<sub>2</sub> penetrates quickly to the depth of the respiratory tract and is absorbed into the blood giving a quick rise to anaesthetic levels. In larger animals the penetration and speed of absorption seem slower and the animals appear disturbed before becoming unconscious.

The Universities Federation for Animal Welfare recommends CO<sub>2</sub> for humane killing of rats, mice and guinea-pigs, but not for larger animals. ROGER EW BANK

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SIR — Dr Freed's letter (*Nature* 11 August, p.482) prompts me to report some results of war-time work on CO<sub>2</sub> intoxication, conducted by a group at the National Institute for Medical Research. The work was virtually unpublished, but an illustrative record of the handwriting of four subjects becoming unconscious while breathing 10, 12.5, 15 and 20 per cent CO<sub>2</sub> in oxygen may be found in *Proc. R. Instn Gt Br.* 41 571-596.

The work arose in connection with shallow-water blackout in divers studying CO<sub>2</sub> accumulation in self-contained breathing sets, possibly due to poor soda-lime canister packing or impaired ventilatory responses during high energy expenditure. Orthodoxy stated at the time (as Dr Freed repeats today) that breathing high concentrations of CO<sub>2</sub> produces an unbearable respiratory stimulation, long before unconsciousness occurs. This is true if air is rebreathed from a bag; then hypoxic and hypercapnic stimuli are combined. If oxygen is rebreathed, however, the hypoxic stimulus is moved, and a different result is obtained. Some respiratory stimulation occurs, rather variable in magnitude from one subject to another, but it is not distressing, and may even be hardly noticed in comparison with the advancing anaesthetic effect of the CO<sub>2</sub>. Particularly striking was the experiment on a very experienced aviation physiologist;

after being subjected to rebreathing from a bag of oxygen, without being told the composition of the respired gas, and having lost consciousness on it, he came round saying "I'll swear that was hypoxia!"

By rebreathing, unconsciousness is lost when the CO<sub>2</sub> concentration has risen to about 10 per cent. If a defined mixture is breathed, some subjects become unconscious on 10 per cent in a few minutes, others require 12.5 per cent. The experience is no more disagreeable than any other form of rapid intoxication, and as with alcoholic intoxication, characteristic differences of behaviour appear at the "endpoint". With longer exposures to concentrations in the range 5-10 per cent, the most striking phenomenon is the strong smell of ammonia on return to fresh air, and the most unpleasant is the "CO<sub>2</sub> off-effect", of headache, pallor, "feeling like death", and nausea and vomiting.

On the particular issue of humane killing, therefore, CO<sub>2</sub> appears to me a very suitable anaesthetic. It was, of course, the first anaesthetic ever used for a surgical operation, in animals by Henry Hill Hickman around 1820.

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## Power in Washington

SIR — Although it does not affect the argument in "Public enterprise and private sector" (*Nature* 11 August, p.473), the Washington Public Power Supply System (WPPS) was not and is not "... the electricity supplying electric power in the northwestern United States ...", but a peculiar entity formed by a number of Washington public utility districts, operating usually at the county or city level, and having contracted with a number of other northwestern utilities, both public and private, for the sale of nuclear generated power from five reactors, three of which have now been discontinued.

The separate utilities' reluctance to pay for the discontinued plants led to court proceedings which ended in findings that the Washington Public Utility Districts lacked the power to form WPPS originally and that many of the other public utilities lacked the power to enter into the kinds of contracts involved — rather more than a simple current purchase of power. Thus the individual utilities are free of liability (pending resolution of the bondholders' suit for liability because of utility board members' alleged civil fraud and negligence) and WPPS has no other sources of revenue sufficient to service the bonds, and has therefore defaulted.

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## Homology redirected

SIR — Hughes (*Nature* 24 March, p. 706) calls attention to the continued misuse of the term *homology*. Mayr *et al.*<sup>2</sup> proposed the term *dendrogram* for phylogenetic schemes based on contemporary forms (vs. "phylogenetic trees" for such schemes based on fossil evidence) but failed to suggest a term to describe the suggested relationships of the components (Mayr<sup>3</sup> still maintains this definition of dendrogram). The inappropriate and erroneous use of *homology* has also troubled me and in 1971<sup>4</sup> I proposed using the available and related term *paromology* (Gr., partial admission, from *παρ* (*α*-subsidary + *ομολογία* agreement, admission, Homology. OED). Thus while feathers and hair are homologous to scales, the chromosomal bands of human, gorilla, chimpanzee and orangutan would be paromologous as would be the various forms of cytochrome *c* (ref.5) and myoglobin<sup>6</sup>. Further, while arms and wings are *homologues*, the *cdc2* gene of *Schizosaccharomyces pombe* and the *cdc28* gene of *Saccharomyces cerevisiae*<sup>7</sup> would be *paromologues* as would be the crozier of the ascomycetes and the clamp of the basidiomycetes.

Many of the relationships that scientists are attempting to express today are based on a wide diversity of contemporary forms. There is an urgent need for an acceptable term to express these imputed relationships that will not erode and debase the meaning of homology. I believe paromology and its derivatives could satisfy this need. It could be applied to comparative behaviours, to similar-appearing fossils until such time as they were historically linked, to comparable human artefacts excavated from different sites, and to the many correlative structures found in genetics, molecular and cell biology, and biochemistry. The introduction and consistent use of the "new" set of terms could markedly clarify the presentation and understanding of these two fundamentally different ways of classifying species: on the basis of similarities supported by fossil evidence<sup>8</sup> or on the basis of similarities alone<sup>9</sup>. This would be particularly true if both classifications were used together.

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# Gradualism, punctuated equilibrium and the *Origin of Species*

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*Darwin's views on the 'tempo and mode' of evolution are examined through six successive editions of the Origin of Species and through unpublished manuscript material. Although Darwin was a gradualist, there was a significant overlap between his views and those of the proponents of the current theory of punctuated equilibrium.*

THE model of punctuated equilibrium has given new impetus to evolutionary thinking, raising significant questions concerning both the nature of the fossil record and the mechanism of the evolutionary process. From the earliest formulation of the theory<sup>1,2</sup>, to its later refinement<sup>3</sup>, and its recent expansion<sup>4</sup>, its authors have provided a stimulating and provocative hypothesis.

One aspect of the resulting discussion of evolutionary theory has been the relationship of the new theory to Darwin's evolutionary views. Proponents of punctuated equilibrium have tended to suggest that Darwin's views in 1859 were limited to a "pure gradualism"<sup>5</sup>.

Stanley<sup>5</sup> has given a similar interpretation of Darwin's views:

"Even if Darwin had possessed theoretical reasons for adopting a punctational model of evolution," he wrote, "for him to have advanced such a scheme would have seemed as absurd as were many of the speculative pronouncements of his predecessors. . . [He] would have been claiming that evolution . . . was operated by a natural mechanism . . . in small, localized, transitory populations."

In contrast, others<sup>6-10</sup> have suggested that Darwin's views were not limited to gradualism, but there has been no detailed analysis of Darwin's views on this topic. Here I review the development of Darwin's views on the tempo and mode of evolution, and in particular, whether "gradualism" in its "strict formulation" was a central theme of Darwin's view, and whether his view of evolution was limited to this particular model.

The distinction between Darwin's own views and those of contemporary proponents of phyletic gradualism is of particular importance, as is the precise meaning of "Darwinism" and the "Darwinian view". This has provoked some lively correspondence<sup>11,12</sup>, but Gould's suggestion<sup>4</sup> that the term "Darwinism" should be restricted to the body of thought allied with Darwin's own theory of mechanism seems the most helpful approach, even though, as Gould has observed, this "does

not provide an unambiguous definition, if only because Darwin himself was a pluralist. . . ."

In such a review, it is also helpful to distinguish two related but distinct aspects of Darwin's work: the first includes his views on the pattern of transformation: the continuity of phyletic gradualism as contrasted with the periodicity of punctuated equilibrium, and the related question of their exclusivity or relative frequency. The second aspect involves the rapidity of change once change occurs, whether it is episodic or continuous. There are two additional aspects of the current debate, to which I have devoted only passing attention in the present paper: Darwin's view of the general mechanisms of transformation, and the adequacy of the fossil record to allow us to distinguish between punctuated equilibrium and phyletic gradualism.

## Darwin's gradualist views

Darwin used the term "gradual" 15 times in the first edition of *The Origin*<sup>13</sup>, the term "gradually" 12 times, and the term "gradation" or "gradations" 47 times<sup>24</sup>. Although some of these references have nothing to do with the origin of species ("the slow and gradual rising of the land," for example) it is clear that Darwin was a gradualist. He wrote of the development of instinct by "slow and *gradual* accumulation of numerous, slight yet profitable, variations" (ref. 13, p.454); of "the geological succession of organic beings" by "their slow and *gradual* modification, through descent and natural selection" (p.312); of the "*gradual* divergence in character of the species descended from a common parent"; of the "*gradual* reduction of various organs, until they have become rudimentary" (p.479); of "the *gradual* modification of parts or organs" (p.479) and so on. In his Recapitulation, discussing the explanation of homologous structures, he invoked "the theory of descent with *slow* and *slight* successive modifications" (p.479). "Species have changed," Darwin wrote, "and are still slowly changing by the preservation and *accumulation of successive slight favourable variations*" (p.480) (all italics are mine).

This view is illustrated by the single figure which Darwin included in *The*

*Origin* (ref. 13, p.117). In the figure the "little fan of diverging dotted lines" denoting "varying offspring" represents a gradualist view, but it is worth noting that it shows no "even" rate of change (compare p.89, ref.2). Darwin assigned these lines the status of a "fairly well-marked variety" when — and only when — they reach one of his horizontal time lines in the diagram, by which time "a sufficient amount of variation is supposed to have been accumulated to have formed a fairly well-marked variety, such as would be thought worthy of record in a systematic work" (ref. 13, p.117).

Darwin estimated the time factor involved in such change. He wrote "The intervals between the horizontal lines in the diagram may represent each a thousand generations; but it would have been better if each had represented ten thousand generations" (ref. 13, p.117). (In the sixth edition (ref. 14, p.91) this becomes "a thousand or more generations".) He also wrote "In the diagram each horizontal line has hitherto been supposed to represent a thousand generations, but each may represent a million or hundred million generations" (ref. 13, p.124). (In the sixth edition (p.95) this became "a million or more generations".) Darwin made it clear (ref. 13, p.120) that the development of sub-species involved the same process as that involved in the development of varieties.

So Darwin was a gradualist, and the gradualism required an interval of one thousand generations for the development of new varieties or species (ref. 13, p.120). So far, so good, so gradual.

## Punctuated equilibrium

But what, exactly, does this gradualism imply? Eldredge and Gould<sup>2</sup> have provided a useful comparison of what they describe as the "Darwinian perspective", called by them "phyletic gradualism", with the newer tenets of "punctuated equilibrium" and its expression in the fossil record. They list the following features:

### Phyletic gradualism:

- (1) New species arise by the transformation of an ancestral population into its modified descendants.
- (2) The transformation is even and slow.

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- (3) The transformation involves large numbers, usually the entire ancestral population.
- (4) The transformation occurs over all or a large part of the ancestral species' geographic range.

These statements imply several consequences, two of which seem especially important to palaeontologists:

- (1) Ideally, the fossil record for the origin of a new species should consist of a long sequence of continuous, insensibly graded intermediate forms linking ancestor and descendant.
- (2) Morphological breaks in a postulated phyletic sequence are due to imperfections in the geological record<sup>2</sup>.

By contrast, in punctuated equilibrium:

- (1) New species arise by the splitting of lineages.
- (2) New species develop rapidly.
- (3) A small sub-population of the ancestral form gives rise to the new species.
- (4) The new species originates in a very small part of the ancestral species' geographic extent — in an isolated area at the periphery of the range.

These four statements again entail two important consequences:

- (1) In any *local* section containing the ancestral species, the fossil record for the descendant's origin should consist of a sharp morphological break between the two forms. This break marks the migration of the descendant, from the peripherally isolated area in which it developed, into its ancestral range. . .
- (2) Many breaks in the fossil record are real; they express the way in which evolution occurs, not the fragments of an imperfect record. The sharp break in a local column accurately records what happened in that area through time (ref. 2, p.96).

If we set aside the question of whether the "modern synthesis" is compatible with punctuated equilibrium<sup>15</sup> and accept Eldredge and Gould's analysis of phyletic gradualism as an accurate description of the views of contemporary advocates, we might then ask whether the same views can be attributed to Darwin. We must ask whether there are tenets of "phyletic gradualism" that he would have rejected and whether there are aspects of what we now call punctuated equilibrium that he would have accepted.

### Darwin's limited gradualism

Darwin's gradualistic model was not exclusive, even though he remarked, in discussing the development of rudimentary organs, "... I doubt whether species under nature ever undergo abrupt changes."<sup>13</sup> Darwin's discussion of the origin of *organs*, however, needs to be distinguished from his discussion of the origin of *species*.

It has been suggested<sup>2</sup> that the "Darwinian perspective" regarded transformation as "even and slow". But Darwin's own writing — even in the first edition of the *Origin* — explicitly denied any such

"even" transformation or continuous change. Darwin wrote: "For we have reason to believe that *only a few species are undergoing change at any one period*" (ref. 13, p.463). And again, "A number of species, however, keeping in a body might *remain for a long period unchanged*" (p.488) (*italics mine*).

Thus, Darwin's gradualistic model also embraced a large component of stasis, an essential part of the current model of punctuated equilibrium.

But Darwin added two other significant qualifications to his interpretation of the simple diagrammatic model of gradual speciation. First he wrote, "... I do not suppose that the process ever goes on so regularly as is represented in the diagram, though in itself made somewhat irregular" (ref. 13, p.118). Second, in a very significant passage, Darwin explained, "The variations are supposed to be extremely slight, but of the most diversified nature; *they are not supposed all to appear simultaneously, but often after long intervals of time*" (p.117) (*italics mine*).

Thus, even in 1859, Darwin did not advocate at least one of the key tenets of phyletic organism that "... transformation is even and slow"<sup>2</sup>. To Darwin, transformation was *not* "even"; whether it was "slow" depends on our definition of speed.

### Mode of evolution

But what of the third and fourth tenets of phyletic gradualism:

- (3) The transformation involves large numbers, usually the entire ancestral population.
- (4) The transformation occurs over all or a large part of the ancestral species' geographic range?<sup>2</sup>

Here again we should let Darwin speak for himself. In reviewing the nature of the fossil record and the "sudden" appearance of new species, Darwin wrote as follows: "One other consideration is worth notice: with animals and plants that can propagate rapidly and are not highly locomotive, there is reason to suspect, as we have formerly seen, that their *varieties are generally at first local*; and that *such local varieties do not spread widely* and supplant their parent-forms until they have been modified and perfected in some considerable degree. According to this view, *the chance of discovering in a formation in any one country all the early stages of transition between any two forms, is small, for the successive changes are supposed to have been local or confined to some one spot*. Most marine animals have a wide range . . . so that with shells and other marine animals, it is probably those which have had the widest range, . . . which have oftenest given rise, first to local varieties and ultimately to new species; and *this again would greatly lessen the chance of our being able to trace the stages of transition in any one geological formation*" (ref. 13, p.298) (*italics mine*).

Although he distinguished between organisms that are "highly locomotive" and those that are not, Darwin seems to have regarded this mode of origin as common to both. A little later, Darwin restated his conclusion: "... analogy leads me to believe that it would be chiefly these far-ranging species which would oftenest produce new varieties; and the *varieties would at first generally be local or confined to one place*, but if possessed of any decided advantage, or when further modified and improved, they would slowly *spread and supplant their parent-forms*. When such *varieties returned to their ancient homes*, as they would differ from their former state, in a nearly uniform, though perhaps extremely slight degree, *they would*, according to the principles followed by many palaeontologists, be *ranked as new and distinct species*" (ref.13, p.301).

It is important to recognize here, as Mayr<sup>16</sup> has emphasized, that Darwin, like his contemporaries, used the term "variety" in two distinct senses, between which he did not always distinguish. Darwin viewed the variety as a transitional form: "... a well-marked variety may be justly called an incipient species" he wrote (ref. 13, p.52). His usage of the term referred, however, not only to individual variants, but also to populations.

In the summary to Chapter X, Darwin repeated his conclusion that "... the *duration of each formation is, perhaps, short compared with the average duration of specific forms; that migration has played an important part in the first appearance of new forms in any one area* and formation; that widely ranging species are those which have varied most, and have oftenest given rise to new species; and that *varieties have at first often been local*. All these causes taken conjointly, must have tended to make the geological record extremely imperfect, and will to a large extent explain why we do not find interminable varieties, connecting together all the extinct and existing forms of life by the finest graduated steps" (ref. 13, p.342) (*all italics mine*). This is a remarkably compact statement of the concept of local, periodic speciation and its reflection in the fossil record.

Darwin has been criticized for the emphasis he placed on the "imperfection" of the fossil record, and certainly he emphasized the hazards of preservation, just as we do today. But Darwin's description of the fossil record as "imperfect" also represents a view similar to that of contemporary exponents of punctuated equilibrium. It explains the absence of transitional fossil forms in a local rock succession, not by the non-preservation of transitional forms that once lived in that particular area, but rather by their never having lived in that area. Transitional forms were restricted in life to another limited part of the species' range, later spreading to the wider population. Paradoxically Darwin, in describing the local fossil record as



"imperfect" invoked — in part — the same explanation as do Eldredge and Gould<sup>2</sup> in describing fossil breaks as "real".

This was not, of course, in Darwin's view, an exclusive explanation of the fossil record. Thus, in the sixth edition of the *Origin* (ref. 14, p.289) he adopted Lyell's metaphor of "the geological record as a history of the world imperfectly kept, and written in a changing dialect; of this history we possess the last volume alone, relating only to two or three countries. Of this volume, only here and there a short chapter has been preserved; and of each page, only here and there a few lines."

But, that view notwithstanding, Darwin clearly regarded local isolation and periodic modification of varieties and their subsequent migration to other areas as major contributory factors to the paucity of the fossil record (ref. 13, p.342).

Three of the four "tenets" of contemporary phyletic gradualism<sup>2</sup> were thus specifically repudiated by Darwin in his writing. The fourth — "New species arise by the transformation of an ancestral population into its modified descendants" — is shared in substance by all those who accept the fact of evolution. Further, both the "two important consequences" of punctuated equilibrium, were accepted by Darwin.

All but the last of the quotations I have given are taken from the first edition of *The Origin of Species*. They provide no evidence for a "lovely tree of pure gradualism that he [Darwin] sketched in the first edition of the *Origin*". Darwin was a gradualist, but he was not a pure gradualist; his views had the elasticity to anticipate most of what have since become our present views of the origin of species.

## Darwin's emphasis on isolation

But Darwin's views on evolution were not static after 1859. They evolved. His unpublished notes and annotations and later editions of *The Origin* contain even more emphatic statements on these topics. It is worth recording that over a period of 13 years, Darwin rewrote and expanded over 75 per cent of the sentences that make up the *Origin*<sup>17</sup>. The sixth edition is almost a third as long again as the first.

Darwin's personal copy of the second edition of *Origin*<sup>18</sup> (1860) has many marginal notes. Some, but not all, of these appear as amendments in the third edition<sup>19</sup>. Some of the changes in the second and third editions reflect Darwin's growing emphasis on the role of isolation and its relationship to time. In the second edition (p.105) Darwin wrote "Although I do not doubt that isolation is of considerable importance in the production of new species. . ." In his copy of the second edition Darwin deleted the words "I do not doubt that," so that the passage in the third edition (ref. 19, p.111) reads "Although isolation is of considerable importance. . ." On the previous page of both the second and the third editions (pages 109, 110 respectively) Darwin stated,

"Isolation, also, is an important element in the process of natural selection". In the third edition, however, he inserted an emphatic new paragraph, which begins "The mere lapse of time by itself does nothing for or against natural selection. I say this because it has been erroneously asserted that the element of time is assumed by me to play an all-important part in natural selection, as if all species were necessarily undergoing slow modification from some innate law" (ref. 19, pp.110–111).

Another small but significant notation in Darwin's copy of the third edition also appears as an amendment in the fourth, where Darwin dealt with the question of major structural change. He saw the same local origin and rapid dispersal being influential. He wrote (ref. 19, p.329) ". . . it might require a long succession of ages to adapt an organism to some new and peculiar line of life, for instance, to fly through the air; and consequently that the transitional forms would often long remain confined to some one region; but that when this adaptation had once been effected, and a few species had thus acquired a great advantage over other organisms, a comparatively short time would be necessary to produce many divergent forms, which would be able to spread rapidly and widely throughout the world." (The italicized portion was added by Darwin in the fourth edition, ref. 20, p.365.)

These examples through the successive editions of the *Origin* suggest that Darwin, though he accepted the significance of geographic isolation in 1859, clarified and emphasized it in the early 1860s. This is confirmed by the marginal notes and published corrections he made in the fourth and later editions of the *Origin*. Thus in the fifth edition (ref. 21, p.120) for example, he writes, "Moritz Wagner has lately . . . shown that the service rendered by isolation in preventing crosses between newly formed varieties is greater even than I have supposed." Although Darwin was convinced that geographic isolation was of "great importance" in the origin of new species, he believed, as Mayr<sup>16</sup> has noted, that it was not essential, and that "largeness of area", "sexual aversion", "divergence" and mutual sterility were also major factors in preventing varieties from "blending". "I can by no means agree. . .," Darwin wrote (ref. 14, p.82), "that migration and isolation are necessary elements for the formation of new species."

Nowhere is Darwin's mature view better illustrated than in his letter of 1876 to Moritz Wagner, ". . . I believe that all the individuals of a species can be slowly modified within the same district, in nearly the same manner as man effects by what I have called the process of unconscious selection . . . I do not believe that one species will give birth to two or more new species, as long as they are mingled together within the same district." A letter of 1878 to K. Semper is even more specific, ". . . There are two different classes of cases, as it ap-

pears to me, viz, those in which a species becomes slowly modified in the same country (of which I cannot doubt there are innumerable instances) and those cases in which a species splits into two or three or more new species; and in the latter case, I should think nearly perfect separation would greatly aid in their "specification", to coin a new word . . . I remember well, long ago, oscillating much; when I thought of the Fauna and Flora of the Galapagos Islands I was all for isolation, when I thought of S. America I doubted much"<sup>22</sup>.

## The tempo of evolution

Some recent writers have suggested that Darwin embraced the idea of stasis and periodic speciation only at the time of writing the fourth edition of the *Origin*. That this was not the case can be seen from the quotations already given from the first edition. Another statement by Darwin in the third edition of the *Origin* (p.139) also clearly anticipated one principle of punctuated equilibrium. In answer to criticism by the "celebrated palaeontologist, Professor Bronn", Darwin wrote "On the erroneous supposition that all the species of a region are believed by me to be changing at the same time, he justly asks how it is that . . . but it is sufficient for us if some few forms at any one time are variable, and few will dispute that this is the case" (italics mine). His belief in rapid replacement did, however, receive added emphasis in later editions (see the fourth edition, p.365).

The back endpaper of Darwin's annotated copy of the fourth edition of the *Origin* includes three brief notes in Darwin's hand. One of these reads

"365 )  
) rate of change."  
359 )

This was presumably an index notation intended for later revisions, although it does not appear in the index of subsequent editions. But it clearly indicates Darwin's own assessment of the significance, not only of the "new" passage on p.359, but also of the statement on p.365 marked by him by a vertical pencil line in the text, which reads ". . . it might require a long succession of ages to adapt an organism to some new and peculiar line of life, for instance, to fly through the air; and consequently that the transitional forms would often long remain confined to some one region; but that, when this adaptation had once been effected, and a few species had thus acquired a great advantage over other organisms, a comparatively short time would be necessary to produce many divergent forms, which would be able to spread rapidly and widely throughout the world". (Italics represent those parts marked by Darwin.) Darwin's manuscript notes thus suggest that his view on rates of change involving major environmental adaptation gave considerable prominence to the factors involved in local speciation, and subsequent rapid development and dispersion. It is also noteworthy that this statement



(fourth edition, p.365) remained essentially unchanged from the first edition (pp.303–304), although Darwin did add the phrase “and consequently that the transitional forms would often long remain confined to some one region”. This further emphasizes the growing importance in his later thinking of geographic isolation in the origin of new species.

The other passage included in Darwin's manuscript note is one new to the fourth edition of the *Origin*, and it illustrates the growing emphasis which he placed on what we should now call “punctuated equilibrium”. Darwin included it as a qualification amongst the “Chief Additions and Corrections” in the list that he provided to accompany the fourth edition (p.xii). “The periods during which species have undergone modification are probably short compared with those during which they have remained unchanged” (p.359). In the text he qualified his earlier statement, “. . . I do not suppose that the process ever goes on so regularly as is represented in the diagram, though in itself made somewhat irregular,” with the telling addition “. . . nor that it goes on continuously; it is far more probable that each form remains for long periods unaltered, and then again undergoes modification” (p.132) (italics mine).

How then are we to interpret Darwin's mature views on the pattern of speciation? They are, I think, a combination of gradualism (small, cumulative, continuous change) and what we now identify as punctuated equilibrium. His prevailing view was one of gradualism, but he also viewed the episodic, local pattern of development as a significant and characteristic component of the evolutionary process.

## Rates of speciation

We have seen that Darwin regarded the “imperfection” of the fossil record as arising from two sources. First he recognized the geological hazards that militate against the probability of the preservation of any individual or group. But he also saw the imperfection of the record as arising, not only from non-preservation of a gradual succession of widespread populations, but from the replacement of a local population by modified forms from neighbouring areas. He accepted the influence of both local, episodic speciation and migration.

The present interpretation of punctuated equilibrium suggests that the rise of new species is most frequently not only episodic and local, but also rapid (as opposed to gradual, widespread, and slow). Is such “rapid” change consistent with Darwin's “gradualist” views? Darwin, as we have seen, thought of new varieties as arising in a period of a thousand or ten thousand generations. A species would, therefore, presumably require ten to a hundred thousand generations. This figure is consistent with that suggested for the rate of speciation ( $5 \times 10^3 - 5 \times 10^4$  years) in Late Cenozoic fresh water molluscs by

Williamson<sup>23</sup>. We need also to bear in mind that when Darwin wrote that the intervals between the lines on his “species diagram” might represent anything from a thousand to a hundred million generations, he presumably included in that interval the time during which a species might “remain for a long period unchanged” (ref. 13, p.488). But slowness is not synonymous with gradualism.

Recent discussions of punctuated equilibrium have tended to stress the rapidity of speciation. Thus Gould and Eldredge<sup>3</sup> write of a “geological microsecond”. But, as Huxley<sup>8</sup> has pointed out, if speciation is to be viewed as a microsecond, then the whole age of the Earth has to be compressed into less than a second, and this is not a very helpful scale. If, on the other hand, one adopts a different scale in which a year is represented by one second, life on the Earth would have existed on that scale for rather more than a century, and  $10^5$  years would be represented by a day<sup>8</sup>. Whether this is “gradual” or “rapid” becomes a matter of definition. But current estimates of the time factor involved show little difference from those suggested — wholly intuitively — by Darwin.

## Rate of evolution of higher taxa

In describing his figure of the development of new species, Darwin wrote “After ten thousand generations, species (A) is supposed to have produced three forms . . . these three forms may still be only well-marked varieties; or they may have arrived at that doubtful category of sub-species” . . . “In the diagram I have assumed that a second species (I) has produced . . . after ten thousand generations, either two well-marked varieties ( $W^{10}$  and  $Z^{10}$ ) or two species, according to the amount of change. . .” (ref. 19, p.126–127). This statement was modified and strengthened in the manuscript corrections to the fourth edition (p.154) by Darwin's deletion of his comment on “the doubtful category of sub-species” and his addition of the possibility of supposing “the steps in the process of modification to be more numerous or greater in amount, to convert these three forms into *doubtful or at last well-defined species*” (italics are Darwin's manuscript insertion). This appears as an amendment in the fifth edition (p.136).

The degree of change which Darwin vis-

ualized as possible in the time span represented by his diagram (14,000 generations) is, perhaps, best summarized by his manuscript notes in the Cambridge copy of the fourth edition (p.137). “Hence the six new species descended from (I), and the eight descended from (A), will have to be ranked as distinct sub-families or families”. The fourth edition text (p.137) referred to them only as “very distinct genera or even as distinct sub-families”. This manuscript change does not appear in the fifth edition (p.140) but Darwin's manuscript note suggests that his estimate of the rate of taxonomic change increased after 1866. The appearance of new families in 14,000 generations is scarcely very slow change in the sense implied by some recent writers, some of whom suggest, for example, that Darwin “. . . would have been shocked at how rapidly small populations can diverge to form new genera”<sup>5</sup>. Part of the confusion here arises from Darwin's own use of such phrases as “extreme slowness”. Clearly, however, his estimates of the rate of change are not significantly different from those of contemporary proponents of punctuated equilibrium.

## Conclusion

The hypothesis of punctuated equilibrium is of major importance for palaeontological theory and practice. Several significant tenets of this view were enunciated by Darwin. Gould<sup>4</sup> was right. Darwin was a pluralist. He was not only a gradualist; his views also overlapped what we should now describe as “punctuated equilibrium”. It would be misleading to imply that he anticipated every nuance of the theory. His views on the importance of transformation of local varieties in the process of speciation and on the pattern and tempo of speciation were clearly stated in the first edition of the *Origin*, though both received a more full and explicit discussion in the fourth edition than in the first.

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# How empty is the vacuum?

*A solution to the problem of radiation from an atom in a loss-free cavity raises questions about the reality of emptiness.*

WHAT happens when an atom in an excited state is allowed to radiate not in a vacuum (the simple case) but in a conducting metallic cavity of some kind, a waveguide for example? On the face of things, this seems the kind of problem that would have been tackled on the back of an envelope by any one of Bohr, Born or Heisenberg more than half a century ago. It is therefore something of a surprise that a group from the University of Rochester in the United States now claims to have made "the first fully quantum mechanical predictions about the spectrum of spontaneous emission into a lossless cavity" (J.J. Sanchez-Mondragon, N.B. Narozhny and J.H. Eberly, *Phys. Rev. Lett.* **51**, 550-553; 1983).

Quite apart from the inherent (and surprising) outcome of the calculation, the paper adequately explains why the problem has not been solved before and may even help to clear up a general confusion about the sense in which empty space is supposed to be filled with "virtual photons" and, for that matter, "virtual gluons", "virtual gravitons" and even the occasional (and "virtual")  $Z^0$  or  $W^\pm$  meson. For Sanchez-Mondragon *et al.* show that the radiation from a single isolated atom in a lossless cavity may be profoundly different from that of a similar atom in free space, which is, after all, only another way of saying that the two regions of vacuum, each of them empty, are nevertheless differently constituted.

The problem has an obvious practical interest in the operation of masers, and the earliest study quoted by Sanchez-Mondragon *et al.* is credited to E.M. Purcell in 1946; understandably that was concerned with the properties of radiation from "atoms" (for example ammonia molecules) in cavities tuned to the characteristic radiation frequency. Interest in what happens far away from resonance was, however, stimulated two years ago by Daniel Kleppner (*Phys. Rev. Lett.* **47**, 233-236; 1981) who argued that an atom whose characteristic radiation frequency is very different from that of the cavity containing it should have an abnormally sharp linewidth and therefore a much longer lifetime against spontaneous radiation than a similar atom in free space. Obviously atoms in such a state would have many practical uses, perhaps in still further improvements of precision frequency standards. But merely checking that linewidths would be narrowed might help to clarify

what is meant by the physical nature of vacuum fields.

That the vacuum of free space should differ in some way from that inside a conducting cavity is natural enough. Classically or otherwise, a cavity will not without difficulty sustain radiation whose wavelength is greater than its own dimensions. In linear waveguides there is always a cut-off frequency  $\nu_0$  such that radiation of lower frequency will not propagate efficiently. If microwaves of longer wavelength do embark along such a waveguide, their energy will quickly be dissipated as ohmic heating in the walls. So much is amply confirmed by everyday experience, but what then will happen if an atom in an excited state with a characteristic frequency below this cut-off is placed in such a waveguide, supposed empty of everything else? The problem is essentially one of quantum mechanics and the result, in the ideal case, is that even if the atom were in an excited state, it could not get rid of its energy by radiation.

This simple (but ideal) conclusion naturally fits well with the picture of radiation processes developed in the past half-century. The system of an isolated atom in an otherwise empty cavity consists of three parts — the atom, the walls of the cavity, and the radiation field of the intervening vacuum with which each of the other components interacts. A photon, being the quantum equivalent of a plane wave or its equivalent, cannot therefore exist if its energy is less than half of the cut-off frequency. But the atom is coupled with the radiation in such a way that the de-excitation of an excited atom is accompanied by the creation of a photon with the same energy in the radiation field. But since the photon cannot exist, the atom cannot radiate.

In reality, as Sanchez-Mondragon *et al.* show, the problem is more complicated. First, it is far from clear what is meant by the spectrum of the radiation that would be generated in these circumstances, for the radiation of a single photon by an atom is not a stationary process, and they are forced to calculate the probability that photons with frequencies in some finite range will be generated. A further difficulty is that approximate solutions will not suffice, and that the shape of the spectrum is likely to differ from that arising with radiation in free space. All this is enough to explain why the problem was not solved in 1925.

The solution now published deals with an atom at the centre of a spherical cavity, and confirms Kleppner's semi-quantitative predictions. In particular, for atoms with characteristic frequencies below the cut-off of the cavity, the spectral lines are narrower than in free space, which entails that the lifetime of the atom is increased. And conversely, if the radiation frequency should be near one of the characteristic resonance frequencies of the cavity, spectral lines will be broadened (or lifetimes decreased).

The surprise, however, is that the calculations show that near resonance (or coincidence of the frequency of atomic radiation with that of a stationary radiation mode within the cavity), the single broadened spectral line splits into two. And if account is taken of the presence within the cavity of pre-existing radiation, the broadened hump of the spectral line is marked by three peaks not symmetrically disposed in frequency. The first splitting into two of the broadened emission line is consistent with quantum electrodynamics. The second, in a cavity not empty of radiation at the outset, is new.

Telling the physical significance of this result will be far from straightforward. The "atoms" considered by Sanchez-Mondragon *et al.* are simple structures, supposed to have only one possible transition frequency so as to avoid the complications of competing radiation processes. So much is fair. But discussion is likely to centre on the mathematical process used for simulating the observation of the spectrum of the radiation from a single atom emitting a single photon into the vacuum within a cavity. The question will be whether Sanchez-Mondragon *et al.* have assumed possible more accurate measurement of the phase associated with a photon than quantum mechanics allows. They promise a later account of the appearance of the third peak in the radiation in terms of phase coherence between the radiating atom and the cavity.

Whatever the outcome of the theoretical argument, the phenomenon should be measurable. Two years ago, Kleppner estimated that it should be possible to detect line-narrowing below a waveguide cut-off frequency by using a 0.4 cm diameter waveguide and, as radiative transitions, those of the Rydberg series of hydrogen-like atoms. Subsequently (see *Nature* **295**, 192; 1982) the effect was measured in the laboratory by Kleppner and colleagues.

**John Maddox**



## Species extinction

# More extinct island birds

from Peter T. Boag

THE peculiarities of birds found on oceanic islands have fascinated biologist and layman alike for centuries. But now curiosity is turning to concern as it becomes clear that island species are going extinct at an unprecedented rate. Only 20 per cent of all bird taxa live on islands, but some 90 per cent of the avian extinctions recorded in historical times have been of island forms<sup>1</sup>. Likewise, although islands make up only a fraction of the Earth's surface, they harbour over 50 per cent of currently endangered bird taxa<sup>1</sup>. The Hawaiian islands are often cited as a prime example of how sensitive island avifaunas are to human interference. But recent palaeontological work by Storrs Olson and Helen James suggests that even in Hawaii, the number of human-caused avian extinctions has been spectacularly underestimated (ref. 2 and see *News & Views* 298, 787; 1982).

Hawaii is one of the most isolated groups of islands in the world, and as a result its flora and fauna display many biological features characteristic of extreme insularity<sup>3</sup>, including high rates of endemism (90–99 per cent for many taxa), with classical examples of speciation in plants, insects and birds<sup>2</sup>. Before Olson and James's investigations, most naturalists had assumed that Hawaiian ecosystems were relatively untouched up to the arrival of James Cook in 1778, and that by combining present-day distributions with historical notes and museum specimens, one could produce a relatively complete picture of the original Hawaiian avifauna<sup>4</sup>.

But in the early 1970s, fossil birds began to be discovered in increasing numbers, largely through the efforts of keen amateur collector Joan Aidem on the island of Molokai. Specimens inevitably found their way to Alexander Wetmore at the Smithsonian Institution, and when Wetmore died in 1978, Olson took over the project. Since then literally tens of thousands of avian fossils and subfossils have been exhumed. New discoveries on the island of Maui last summer and a backlog of identification mean that the list of extinct forms will grow, but already the results have more than doubled the number of endemic species known from the archipelago, and have shown that more extinctions occurred at human hands before Cook's arrival than after (Table 1). Some 50 per cent of the original bird species were lost in prehistoric times, while the much publicized extinctions in historic times involved only some 15 per cent of the original species. A similar reduction took place in the number of island populations of endemic landbirds, from at least 137 in prehistoric times to 78 in historic times and 64 at present. A corollary of these findings is that the minimum

number of colonization events required to explain the evolution of Hawaiian endemics must be at least double earlier estimates, up to 20 or more.

Among the more impressive finds were a large number of nonpasserines and corvids, partly explaining the impoverishment of the present avifauna with respect to larger landbirds, and some otherwise unexplained distributional gaps. The extinctions include no fewer than seven species of geese (mostly flightless), two flightless ibises, a sea eagle and small hawk, seven flightless rails, three owls, two crows, a second endemic honeyeater and at least 15 species of Hawaiian finches or

**Table 1** Estimated numbers of endemic land-bird species on the entire Hawaiian archipelago at present, in historic times (~1778) and in prehistoric times (~AD 400)

| Group          | No. of endemic bird species |          |             |
|----------------|-----------------------------|----------|-------------|
|                | Present                     | Historic | Prehistoric |
| Nonpasserines  | 4                           | 4        | 26          |
| Drepanidini    |                             |          |             |
| only           | 19                          | 27       | 42          |
| All passerines | 25                          | 37       | 55          |
| Total          | 29                          | 41       | 81          |

Based on Olson and James's<sup>2</sup> taxonomic assumptions and data, which exclude several forms considered to be endemic subspecies.

Drepanidini. The distribution of the fossils also indicates that many surviving species, now restricted to inaccessible patches of high-altitude forest, were previously common and widely distributed.

It is generally agreed that the main reasons for the disappearance of Hawaiian endemics in historical times were habitat destruction and introduced pests such as the arboreal roof rat (*Rattus rattus*) and disease-carrying mosquitoes (*Culex pipiens*)<sup>5,6</sup>. Olson and James argue that similar processes probably led to prehistoric extinctions. Archaeologists now suggest that after Polynesians colonized the islands between AD 400 and 600, their populations expanded to equal or exceed those seen on some islands even today<sup>2</sup>, and their agricultural practices almost completely eliminated lowland forest from many islands, with evidence of fields and other artefacts up to 900 m above sea level<sup>7,8</sup>. The Polynesians were also efficient hunters, and brought with them domestic mammals such as dogs and pigs. Many of the larger or flightless species of birds would have been quickly eliminated by the combined efforts of human and introduced mammalian predators. The Polynesian practice of using brightly coloured feathers for decorative purposes no

doubt contributed to the demise of endemics unfortunate enough to have showy plumage. Several sites have been excavated which show evidence of extinct species in middens or fire hearths, arguing for the direct effects of human predation. Such sites have proved useful in documenting the contemporaneity of the extinct birds and Polynesians, and this together with some initial radiocarbon dating suggests that many of the fossil and subfossil deposits are unlikely to pre-date the Polynesian invasion of Hawaii. Thus Polynesians had at least as great an impact on Hawaiian ecosystems as did later European arrivals, contrary to the widespread belief that Polynesians affected Hawaii insignificantly compared with their well documented extirpation of New Zealand endemics such as the giant moas (*Dinornithidae*)<sup>9</sup>.

One immediate consequence of these findings is that they call into question the validity of earlier biogeographical analyses of the Hawaiian flora and fauna, and perhaps other Pacific islands as well. For instance, Juvik and Austring<sup>10</sup> noted a strong correlation between the number of endemic Hawaiian birds on islands and island areas, and suggested this was not due to an equilibrium between immigration and extinction<sup>11</sup>, but instead represented 'species-area saturation through adaptive radiation in evolutionary time'. Olson and James correctly dismiss such an explanation, as the new data on prehistoric extinctions clearly show that the avifauna has been nowhere near evolutionary saturation in recent centuries. Despite growing disenchantment with island biogeographical theory<sup>12</sup>, however, it seems premature for Olson and James to ignore the strong empirical relationships between present-day or historical endemic bird species numbers and island areas. The correlations break down when one tries to incorporate the prehistoric data, but this is not surprising given that the fossil analyses remain incomplete.

An obvious alternative explanation for the species-area relationships is area-dependent extinction. Larger islands would have more refuges for threatened species because of their higher altitudes and greater ecological diversity<sup>10</sup>: both people and pests have difficulty

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penetrating highlands. Hawaii may thus provide island biogeographical data similar to 'nonequilibrium' situations such as 'mammals on mountaintops' in the southwestern USA<sup>13</sup>. There islands of boreal habitat were formed on isolated mountains when Pleistocene glaciers retreated; the numbers of small mammal species on these habitat islands seem best explained by an island biogeographical model dominated by area-dependent extinction in the absence of significant immigration or *in situ* speciation. Furthermore, the birds most prone to extinction in Hawaii have been those predicted for area-

dependent extinction in nature reserves: large, or ecologically specialized species, or species with low powers of dispersal<sup>14</sup>. As lowland tropical rainforests around the world continue to disappear and become fragmented, it is ironic that an archipelago which has provided some of the most spectacular examples of speciation may now provide quantitative data on the rates at which species are likely to go extinct in mainland nature reserves. □

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## Genetics

# Control of chromosome behaviour in yeast

from Virginia A. Zakian

EUKARYOTIC chromosomes are transmitted in a highly ordered manner during both mitosis and meiosis. For example, during each mitotic division a yeast chromosome is lost from only about 1 in  $10^5$  cells<sup>1</sup>. Three structures are known to be required for efficient maintenance and inheritance of eukaryotic chromosomes: (1) origins of DNA replication which allow duplication of chromosomes; (2) centromeres, which serve as sites of attachment for spindle microtubules and are required for segregation of chromosomes; and (3) telomeres, the physical ends of chromosomes which are in some way essential for the integrity of linear chromosomes. Until recently, these three structures were studied primarily by indirect procedures. Now, however, the ability to transform yeast with exogenous DNAs allows the functional cloning of sequences with the phenotypes expected for origins of DNA replication, centromeres and telomeres. This ability to clone structural domains of chromosomes has made the analysis of chromosome mechanics in yeast accessible to the most up-to-date techniques of molecular biology.

Three types of sequence have been used in recent experiments. ARSs (autonomously replicating sequences) are identified by their ability to promote extrachromosomal replication of plasmid DNAs<sup>2</sup>, CENs (centromeres) enable replicating plasmids to segregate more efficiently during both mitosis and meiosis<sup>3</sup>, and telomeres allow plasmids to be perpetuated as linear molecules<sup>4</sup>.

One approach to understanding chromosome behaviour is to construct small artificial chromosomes *in vitro* containing ARSs, CENs and telomeres, as well as a structural gene to allow their selection after transformation into yeast<sup>5</sup>. Although these small (10–15 kilobases[kb]) linear CEN plasmids can be maintained during both mitosis and meiosis, their mitotic

stability is markedly less than that of a corresponding circular CEN plasmid<sup>5,6</sup>.

Experiments reported in a recent issue of *Nature* indicate that the instability of these artificial linear chromosomes is due, at least in part, to their small size<sup>6</sup>. Artificial linear chromosomes ranging in size from 10 to 56 kb were constructed by the addition of phage  $\lambda$  DNA. After correction for copy number, the largest of these chromosomes, a molecule about one-third the size of the smallest yeast chromosome, displayed a mitotic stability very similar to that of small circular CEN plasmids<sup>6</sup>. Thus, the instability of linear CEN plasmids, an instability which seems to be a direct consequence of linearization<sup>5,6</sup>, can be remedied by increasing their size. However, even the 56-kb artificial linear plasmids are unstable in mitosis compared with a *bona fide* yeast chromosome<sup>6</sup>.

An alternative approach to studying the biology of whole chromosomes is to introduce or delete specific segments of structural DNA in natural yeast chromosomes and ask what effect these changes have on chromosome behaviour.

Clarke and Carbon<sup>7</sup> used the procedure of fragment-mediated transformation<sup>8</sup> to alter the centromere region of chromosome III. (This technique was also used by Murray and Szostak<sup>9</sup> to construct their large artificial chromosomes.) Three types of alteration were introduced into the centromere region: deletion of CEN3 sequences, inversion of CEN3 relative to flanking sequences and substitution of CEN11 for CEN3 DNA. Each alteration also involved addition to the centromere region of both the structural gene *URA3* (thereby providing a selectable marker for the altered chromosome) and a small portion of pBR322 DNA.

Clarke and Carbon found that deletion of a 627-base pair (bp) CEN3 fragment produces an extremely unstable

chromosome, which can be maintained only in diploid cells growing under selection for expression of *URA3*. In contrast, inversion of CEN3 or substitution of CEN11 for CEN3 has no measurable effect on the mitotic behaviour of chromosome III (determined from rates of chromosome loss, gene conversion and recombination) and very little, if any, effect on chromosome behaviour in meiosis.

These results provide molecular evidence which corroborates conclusions formerly based on cytological experiments (for example, refs 9, 10); that is, that neither inversion nor replacement of a centromere alters its pairing specificity. Thus, although a centromere is required for efficient segregation, it does not function in homologue recognition. Moreover, since the terminal regions of most or all yeast chromosomes share a similar sequence content and organization<sup>11</sup>, it seems unlikely that telomere interactions could direct homologue pairing in meiosis. Rather, other unidentified structures must be responsible for homologue recognition. Since the stability of chromosomes with altered centromeres is indistinguishable from that of a wild-type yeast chromosome, all the structures responsible for duplication, pairing and segregation of yeast chromosomes must be intact and functioning on these molecules<sup>7</sup>. In contrast, the best artificial chromosomes are still  $10^2$ – $10^3$  times less stable than natural yeast chromosomes. The instability of these artificial chromosomes may be caused by any of a variety of factors, for example, the presence of foreign DNA, less than optimal size, or improper spacing of structural domains.

It is also possible, however, that as yet unidentified structures exist that influence the fidelity of DNA replication, chromosome pairing, or chromosome segregation. With the advent of transformation systems which make use of visual assays for plasmid stability<sup>12,13</sup>, this hypothesis can be tested readily by systematically scanning the yeast genome for fragments of DNA which influence the segregation of artificial chromosomes. □

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## Molecular biology

## Eukaryotic genes—are they under torsional stress?

from David M. J. Lilley

In the last few months the answer to the question posed in the title has changed from a reasonably emphatic 'no' to a more intriguing 'maybe'. In this article, I shall attempt to explain the experimental grounds for this shift in thinking.

But first of all, what is meant by torsional stress? By virtue of its double-helical nature DNA may be torsionally deformed rather in the manner of a helical spring, and many natural DNA species are found in an underwound condition, that is, they are twisted in a sense opposite to the right-handed screw of the Watson-Crick helix. The free energy inherent in this alteration of twist is not necessarily available in this torsional form, however. Topological theory shows that if DNA is wound around a cylinder to form a left-handed toroidal coil, then this is equivalent to torsional underwinding. In a pure circular DNA species there is free equilibrium between torsional deformation (twist) and axial winding (writhe) according to a simple mathematical relationship<sup>1</sup>. If however the DNA is fixed to a cylindrical former as a left-handed toroid, then the energy required to impart this deformation is not available as torsional stress. In topological parlance, the DNA exhibits reduced linkage but is unable to enjoy free partition between torsional twisting and tertiary writhing.

In bacteria it is fairly certain that this free partition is at least partially present. Thus prokaryotic DNA is found underwound, that is, negatively supercoiled in a manner unconstrained by protein binding. This supercoiling is introduced enzymatically in an energy-requiring process, as a result of which the molecules are torsionally stressed. This 'extra' energy in the system may be coupled to structural transition in the DNA, and to the formation of open complexes with RNA polymerase to promote transcription.

In eukaryotes the situation is much less clear. Eukaryotic DNA is complexed with an equal weight of histone protein to generate a structure called chromatin. The basic unit of chromatin structure is the nucleosome, crystallographic studies of which<sup>2</sup> have shown that the DNA is wound as a left-handed toroidal coil on a histone core. This is just the situation described above where the inherent underwinding of the DNA is constrained to exist as pure writhing. Torsionally the DNA may well be relaxed for as long as the nucleosome structure remains intact, although if the histones are removed, then there is free partition and the DNA becomes torsionally stressed. In general the prevailing concept

of eukaryotic chromatin has been one of DNA which is wound into nucleosomal and higher-order structures so as to leave it with no overall torsional stress. Early experiments with intercalating probes<sup>3</sup> confirmed that eukaryotic DNA was not under significant torsional stress, although a small but perhaps interesting exceptional sub-component could not be excluded by such studies.

Three recent observations make these assumptions worth a closer examination. Harland *et al.*<sup>4</sup> have presented an elegant demonstration of the profound effects of circularity on transcription from the herpes simplex virus thymidine kinase and adenovirus major late promoters injected into frog oocytes. By measuring the extent of accurate initiation only, these authors demonstrate a 500–1,000-fold increase in template activity of circular plasmids as compared with their linear counterparts. Moreover, this 'switch' may be seen *in ovo* by injection of restriction enzymes which linearize the plasmid inside the oocyte itself, whence transcription reverts to the linear level.

Luchnik *et al.*<sup>5</sup> have also shown that a small proportion of simian virus 40 (SV40) mini-chromosomes extracted from infected monkey cells may be relaxed by topoisomerase I, implying that the DNA is torsionally stressed in a freely partitioned manner. Topoisomerase I is an enzyme which introduces a transient break into the DNA ribose-phosphate backbone which then acts as a swivel point, allowing the dissipation of torsional stress by helix rotation before resealing the broken phosphodiester bond. This process is passive, however, and if the molecule is not torsionally stressed initially then clearly it cannot be relaxed by this treatment. They go on to associate this topologically unrestrained fraction with transcriptionally active SV40 chromatin.

Finally, a third striking and highly relevant observation comes from Nasmyth and co-workers<sup>6</sup>. Stored yeast mating-type gene copies are normally silent, despite the possession of functional promoters, subject to the positive repression by four *SIR* genes. By transforming yeast plasmids containing the nominally silent *HML $\alpha$*  gene into *SIR<sup>+</sup>* or *SIR<sup>-</sup>* yeast, Nasmyth has demonstrated that the reactivation in the *SIR<sup>-</sup>* background is accompanied by a pronounced alteration of plasmid topological state. In this instance there is thus a direct correlation between gene activity and DNA linkage properties.

In summary, then, there are data indicating the importance of circularity in

transcription, the release of torsional constraint into the double helix in a sub-fraction of minichromosomes and the correspondence of gene activity and topology.

Whilst none of these data are open to unique interpretation, their collective significance is thought-provoking and worthy of a little speculation. There is a clear implication that during gene activation the free energy held in the form of the tertiary folding of the DNA about the nucleosome core may be released into the DNA helix itself as torsional stress. Eukaryotic chromosomes are known to be organized as centrally constrained loops or domains<sup>7</sup> which act as independent topological units. Since the bulk of any given loop is probably organized as a solenoid of nucleosomes<sup>8</sup>, any local structural disassembly (for example, loss of histone, although less drastic structural rearrangements with the same topological consequences can be envisioned), at a specific gene for instance, leaves the DNA torsionally stressed at that location. Actually implicit in this statement is the assumption that the nucleosome effectively prevents the transmission of torsional deformation, and whilst this seems highly probable it is currently being tested in a number of laboratories. This also leaves the possibility that structural alteration elsewhere in the domain may also exert torsional effects. One such potential effector is an alteration in the handedness of the DNA helix at some location, formation of Z-DNA<sup>9</sup> being the obvious contender. Potential Z-forming sequences may be abundant in eukaryotic genomes<sup>10</sup>, and anti-Z-DNA antibodies react strongly with *Drosophila* chromosome squashes giving very distinct interbanding staining<sup>11</sup>. The most recent observation in this regard is the presence of potential Z-DNA segments in transcriptional enhancers of several tumour viruses, which bind the anti-Z-DNA antibody when sufficiently highly

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supercoiled<sup>12</sup>.

Probably the greatest argument against the existence of free underwinding in eukaryotic cells is the very large levels of topoisomerase I activity usually found, which might be expected rapidly to relax to equilibrium any such region. But while nucleosome-topoisomerase associations have been isolated from nuclei<sup>13,14</sup>, there exist no data on the possible sub-nuclear location of this enzyme. It might therefore be premature to conclude that eukaryotic DNA could not become stressed, perhaps transiently.

Acquisition of torsional stress by selected parts of the genome may be important for two main reasons. By this means free energy can be released into the DNA in an available manner, which could be used to drive structural transitions and/or promote transcription. Several examples of supercoil-driven DNA perturbation are known, such as cruciforms<sup>15-17</sup> and Z structure<sup>18,19</sup>, and it remains entirely possible that such structural polymorphism is important for gene function. Particularly

intriguing in this respect are the recent observations by Larsen and Weintraub<sup>20</sup>, of nuclease-hypersensitive domains of chick globin gene DNA in supercoiled, but not relaxed, plasmid clones, in positions corresponding to those activated in chromatin. The coupling of supercoiling free energy and transcription has a clear precedent in the prokaryotes. Open complex formation is expected to be stimulated by negative supercoiling, and bacterial promoters exhibit differential sensitivity of promoter strength upon template underwinding<sup>21</sup>. Indeed, the role of supercoiling in the control of prokaryotic gene expression appears to be gaining more and more importance. Whilst the available data for the eukaryotes must still be regarded as preliminary, it is tempting to suppose that similar topological gene control may be important, and further developments in this area are eagerly awaited. □

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## Atmospheric chemistry

# The oceans and the global sulphur budget

from Suzanne Turner and Peter Liss

GASEOUS sulphur compounds, such as sulphur dioxide (SO<sub>2</sub>), dimethyl sulphide (DMS), carbonyl sulphide (COS), carbon disulphide (CS<sub>2</sub>) and hydrogen sulphide (H<sub>2</sub>S), are thought to be important in two main aspects of atmospheric chemistry. First, they affect the acidity of rain directly; SO<sub>2</sub> forms sulphurous and sulphuric acids, and COS produces a solution of the weak acids H<sub>2</sub>S and H<sub>2</sub>CO<sub>3</sub>. Several other sulphur gases (DMS, for example) are also readily oxidized in air to SO<sub>2</sub>, and so further contribute to rainwater acidity. Second, these compounds are reactive in the atmosphere and susceptible to attack by hydroxyl radicals, although the rate of reaction varies between the various compounds<sup>1</sup>. Ultimately, their oxidation leads to the production of sulphate aerosol particles, especially in the stratosphere, where they play an important part in controlling the radiation balance and hence the climate of the Earth<sup>2,3</sup>.

In order to assess quantitatively the role of sulphur gases in these two processes, their global budgets must be understood, that is, the various sources and sinks of the gases must be properly quantified. Such budgeting is made complicated by the perturbation of the natural cycles that results from human activity. For example, about 65 × 10<sup>12</sup> g of sulphur, mostly as SO<sub>2</sub>, are injected into the atmosphere each year from the burning of fossil fuels and the roasting of metal sulphides ores. This is a rather substantial amount relative to the

total natural sources of sulphur to the atmosphere of approximately 80 × 10<sup>12</sup> g per year.

An important term in all global sulphur budgets is the flux of gaseous sulphur across the sea surface. Early budgets always had to invoke a substantial flux of volatile sulphur from the oceans into the atmosphere to achieve balance, and the form of the sulphur was generally assumed to be H<sub>2</sub>S. However, except in very shallow coastal waters overlying reducing sediments, the existence of H<sub>2</sub>S near the sea surface is most unlikely because it is rapidly oxidized to sulphate in oxygenated waters.

In the last few years it has become possible to determine directly several natural sulphur gases in surface seawater, at concentrations of less than 1 part per 10<sup>9</sup> by volume, using gas chromatographic separation and a flame photometric detector. By this approach, it has been shown that relatively high concentrations of DMS are present in oceanic surface waters<sup>4</sup>, and this is related to the productivity of marine algae. The concentration in the atmosphere is, however, considerably lower and the residence time is only about 1 day, owing to the high reactivity of the compound. Applying model calculations, a sea-to-air flux of about 40 × 10<sup>12</sup> g per year has been proposed<sup>4</sup>. DMS therefore can account for about 50 per cent of natural emissions. Clearly the oceans are a significant source of atmospheric sulphur.

Results for COS have shown that it is the most abundant sulphur-containing gas in the atmosphere (512 parts per 10<sup>12</sup> by volume; ref. 5). There is little difference between Northern and Southern Hemisphere values<sup>5</sup>, suggesting that the sources of COS are distributed uniformly; that is, due to slow interhemispheric mixing, a higher concentration in the north could indicate industrial sources. Several sources of COS have been proposed including biomass burning, volcanoes, salt marshes and the combustion of fossil fuels, but the importance of these sources is not yet certain. Hydrolysis of COS in the ocean<sup>6</sup> and reaction with hydroxyl radicals in the atmosphere<sup>2</sup> have been proposed as sinks. The residence time of COS in the atmosphere has been determined by modelling, but as knowledge of the sinks of the gas is limited, estimates vary from 160 days (ref. 2) to 4–7 years (ref. 6).

Recent measurements of COS in oceanic surface waters, in fact, indicate that seawater is almost certainly a source, rather than a sink, of the gas for the atmosphere<sup>7,8</sup>. If hydrolysis is significant in removing COS and production is low, surface waters should be undersaturated with respect to the atmosphere. Conversely, if production is high, supersaturation of the surface seawater will occur. Ferek and Andreae<sup>9</sup> recently published findings for the degree of saturation in ocean waters. Averaging the results for coastal, open-ocean and productive waters, they derive a supersaturation value of about 2. A positive correlation between COS and DMS concentrations was found, suggesting that the presence of COS may also be related to algal productivity. The authors calculate a flux of COS from the oceans to the atmosphere of 0.46 × 10<sup>12</sup> g of sulphur per year. Although this is significantly less than the emission of DMS from the oceans, it shows again that seawater is a considerable source of volatile sulphur to the atmosphere.

In the future it will be necessary to ascertain how the degree of supersaturation of DMS and COS in the oceans varies both spatially and temporally, as well as to identify other sulphur gases which may contribute to the air-sea transfer of this element. □

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## Geochemistry

# Mantle heterogeneity and convection

from H. Bougault, S. C. Cande, J.-G. Schilling, D. L. Turcotte and P. Olson

THE nature of mantle heterogeneity and its geodynamic significance was the subject of a recent symposium\* catalysed by the presentation of the first on-shore results of IPOD Leg 82 of the *Glomar Challenger* drilling. The Leg had been designed to study in time and space mantle heterogeneity beneath the North Atlantic around the Azores.

The symposium was intended to determine what constraints could be placed on convective processes in the mantle from the growing amount of data on incompatible trace elements and isotopic ratios of Pb, Sr, Nd, Hf and rare gases (He, Ar, Xe) in mantle-derived rocks. Geochemical tracers can provide information on mantle heterogeneity that cannot be had from geophysical observations such as gravity field, mean heat flow, deviation in the square root of time law for seafloor elevation, or seismic wave velocities. Their distribution, as observed in mid-ocean ridge basalts (MORB) and in ocean island volcanics (hotspots), gives an integrated picture of the mixing and differentiation going on within the Earth's mantle, and provides clues to the number, geochemical nature and mean age of reservoirs present in the mantle.

The potential for resolving dynamic processes in the mantle has attracted much attention to this infant subject. The important questions at this stage are: what do mantle heterogeneities look like, how do they vary in time, how do they interact with each other and how were they formed?

Isotopic ratios are important indicators of the sources of crustal rocks. Perhaps the most striking fact to emerge from the meeting was that anomalies in isotopic ratios from mid-ocean ridges and island basalts can span an enormous range of spatial scales, and their temporal variability can be short as well as long term. Indeed, the first requirement for any unified model of mantle sources of basalts is that it be able to account for this broad spectrum of spatial and temporal scales.

Results from IPOD Leg 82 illustrated such variability of scales particularly well. The purpose of the Leg was to document when and how the large-scale isotopic gradient (> 800 km long) extending along the present axis of the Mid-Atlantic Ridge (MAR) from the Azores Platform to the Hayes fracture zone developed; whether the Hayes fracture zone was always a boundary between the incompatible element-enriched Azores mantle domain and the depleted source of MORB; and whether the

narrow (100–200 km) spike-like geochemical anomaly now present at 35°N (Oceanographer fracture zone) and superimposed over the large-scale gradient is a permanent feature, or simply spurious.

Samples were taken from a coarse grid (the best there is so far, however) of nine holes covering three isochrons (~ 35 Myr, 18 Myr and zero age) and four spreading flow lines at the latitude of the Azores, FAMOUS, 35°N and south of the Hayes fracture zone. Results presented by the French, British and US laboratories show that south of the Hayes fracture zone the mantle source of MORB has retained its depleted character over the last 35 Myr, thus further confirming the first-order uniformity, or well mixed, nature of this world-wide reservoir. In contrast, lavas derived from both the light rare-earth-enriched and radiogenic Sr- and Pb-rich (Nd-poor) source and the depleted MORB source were observed within a single hole (558) which sampled a time span of only 100–100,000 yr.

The group from the University of Rhode Island emphasized that such local variability is characteristic of hotspots that are no longer centred on the ridge axis, and may reflect the poor mixing conditions resulting from flow along sublithospheric channels extending between the near-ridge hotspots and migrating ridges, which act as sources and sinks of mantle materials, as proposed by Morgan in 1978. The 35°N anomaly, which appears to have persisted over the last 35 Myr sampled, could represent such a case and be associated with the long activity of the New England hotspot. This possibility can fit kinematic reconstructions of the migrating MAR axis relative to fixed North Atlantic hotspots presented by R. Duncan (Oregon State University) and S. Cande and co-workers (Columbia University). There is also general agreement that the Azores mantle plume must have reached the Earth's surface within the last 20 Myr, judging from kinematic reconstructions, the lack of a geochemical anomaly at the latitude of the Azores until 16 Myr and the absence of a down-ridge large-scale isotopic gradient in the oceanic crust of 35–10 Myr age sampled.

In general the on-shore analysis of trace elements (rare earths, for example) and isotopic ratios reported on Leg 82 confirm the conclusions regarding mantle sources and degree of mixing that were reached on-board ship from trace element patterns measured by XRF analyses (for example Nb, Zr, Ti, Y, V). There are, however, two notable exceptions. First, in hole 558 one light rare-earth-depleted lava horizon had

a relatively low  $^{143}\text{Nd}/^{144}\text{Nd}$  ratio comparable to the light rare-earth-enriched source that otherwise characterizes this hole. The discrepancy was interpreted by Jenner and co-workers (Max Planck Institute) as suggesting a recent depletion event, perhaps by fractional or dynamical melting. Second, hole 558 is enriched in radiogenic Pb to a similar degree as the present Azores mantle source, whereas the rare-earth pattern and Nd and Sr isotopes show the source of basalts from this entire hole to be MORB-like. No explanation was provided for this discrepancy.

The two exceptions emphasize both the need for analysis of various isotopes and trace elements in the same samples, and the power of the geochemical approach in providing constraints on the timing of enrichment events in the mantle. This was also well illustrated by the correlation of  $^{129}\text{Xe}/^{130}\text{Xe}$  with  $^{134}\text{Xe}/^{130}\text{Xe}$ ,  $^{40}\text{Ar}/^{39}\text{Ar}$  and  $^3\text{He}/^4\text{He}$  presented by Staudeger and Allègre (University of Paris) which suggest that the Earth's mantle must be more than 99 per cent degassed; the depletion of the MORB source must have taken place within some 80 Myr from the time the Earth accreted; and mixing between blob sources and the MORB source must have taken place recently.

Fine-scale local mantle source variability was best illustrated from data on intraplate island chains in the Pacific. For example, from Pb, Sr, Nd and Hf isotopic variability among basalts from the Hawaiian–Emperor Chain, the USGS team at Denver recognized six distinct groups. Episodic variability of sources over the 30,000-yr time span of the single Mauna Loa volcano, Hawaii, was also documented by J. Rhodes (University of Massachusetts), and the entire spectrum of world oceanic variability in Pb, Sr and Nd isotope ratios was observed among the French Polynes-

## 100 years ago

### A PLEA FOR PURE SCIENCE

I am required to address the so-called Physical Section of this Association. Fain would I speak pleasant words to you on this subject; fain would I recount to you the progress made in this subject by my countrymen, and their noble efforts to understand the order of the universe. But I go out to gather the grain ripe to the harvest, and I find only tares. Here and there a noble head of grain rises above the weeds; but so few are they that I find the majority of my countrymen know them not. We must consider what must be done to create a science of physics in this country, rather than to call telegraphs, electric lights, and such conveniences by the name of science. I do not wish to underrate the value of all these things: the progress of the world depends on them, and he is to be honoured who cultivates them successfully. And yet it is not uncommon to have the applications of science confounded with pure science; and some obscure American who steals the ideas of some great mind of the past and enriches himself by the application of the same to domestic uses, is often lauded above the originator of the idea.

Condensed abstract of the address of Prof. H.A. Rowland of Baltimore before the American Association at Minneapolis.

From *Nature* 28, 510, 20 September 1883.

\*The spring meeting of the American Geophysical Union was held in Baltimore on 31 May–3 June.

sian Islands reported by P. Vidal and co-workers (University of Chermond-Ferraud). The apparent multiplicity of isotopic sources for intraplate hotspots is not entirely surprising since additional complexity is expected to arise in the interaction and mixing of magmas from various mantle sources with old lithosphere which has been altered by seawater-derived hydrothermal activity, and sedimentary products of various provenances. Furthermore, longer magma residence times in the lithosphere expected for intraplate volcanism are likely to enhance dispersion in elemental abundances by fractional crystallization. Subsequent mixing of such differentiated melts derived from distinct sources will also enhance scatter in mixing correlations between two isotope ratios. This effect was demonstrated by Verma and co-workers (University of Mexico) by comparing scatter in mixing trends in Sr and Nd space for lavas from the Galapagos Islands and the nearby spreading centre.

The identification of various end-member reservoirs, of which other reservoirs may be a mixture, was also discussed at length. Zindler (Columbia University) further stressed the need to recognize three end-member reservoirs in terms of Pb–Sr–Nd isotopic space (*Nature* **298**, 519; 1982). The three reservoirs comprise two of hotspot type: the St Helena, also reported to be present beneath Tubuai Island in the Pacific by Vidal, and the Tristan d'Acunha; and, of course, the depleted MORB source. Allègre showed on the basis of relative dispersion of  $^{87}\text{Sr}/^{86}\text{Sr}$  that the depleted MORB source is more contaminated by injection of hotspot-type material beneath the MAR than the more rapidly spreading East Pacific Rise. Independently, Craig (University of Southern California) pointed out two extreme hotspot mantle source types relative to the MORB source: a low  $^3\text{He}/^4\text{He}$  which may correspond to the Tristan type in Nd–Sr–Pb space (for example, Azores, Erebus, Easter, Grand Comore and Galapagos) and which is also found along island arcs and a high  $^3\text{He}/^4\text{He}$  type (for example, Iceland, Hawaii, Reunion, McDonald Seamount, Samoa Island) which does not correspond to any end-member identified in term of Nd–Sr–Pb isotopic space. This raised the question that rare gases may be decoupled from the mantle reservoirs identified with Nd–Sr–Pb isotopes, and may be migrating independently within the mantle, perhaps in association with metasomatic fluids — a mechanism which received direct mineralogical and isotopic support from the study of various peridotites by S. Haggerty (University of Massachusetts), Hawkesworth and co-workers (Open University, UK) and S. Hart and co-workers (Massachusetts Institute of Technology).

It was clear from the meeting that the distribution of the various reservoirs, how they interact and mix, and their origin are continuing problems. The following ques-

tions were raised in one form or another. Is the mantle layered and convection confined to such layers, or are the enriched mantle domains passively and randomly distributed within the depleted MORB source as emphasized by Davies (Washington University)? Do interactions between layers take place only at the interface between the layers, such as by entrainment by convective eddies from one reservoir to another as suggested by the fluid dynamic experiments of P. Olson (Johns Hopkins University) or is the interaction taking place by buoyant heat-producing element-enriched blobs which penetrate the depleted asthenosphere source of MORBs as emphasized by J-G. Schilling (University of Rhode Island), G. Thompson (Woods Hole Oceanographic Institute) and Allègre and co-workers? Do some of the enriched-mantle-type domains reflect either deeply buried, previously untapped mantle reservoirs as implied by the rare-gas

isotopic data; or reinjection into the mantle of (recycled) continental crust material (De Paolo, University of California, Los Angeles); or subduction of aged lithosphere which would penetrate the 650-km discontinuity (D. Yuen and U. Christensen, Arizona State University and Max Planck Institute); or combinations of these? Are small incompatible element- and volatile-enriched mantle domains embedded in the more depleted MORB source preferentially melted as they rise towards the Earth's surface (N. Sleep, Stanford University)? No unified model emerged from the meeting, but what was achieved was a better sense of where the ambiguities lie and what the important questions will be in the future. □

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## Mineralogy

# The epidote jigsaw

from Colin Graham

MINERALS of the epidote group [ $\text{CaAl}_2(\text{Al,Fe})\text{Si}_3\text{O}_{12}(\text{OH})$ ] are stable throughout the Earth's crust, from the shallow levels of geothermal systems to the depths of the lowermost crust or upper mantle. They are widely distributed in metamorphosed basaltic and calcareous rocks, and their occurrence and stability are sensitive to both temperature and pressure, as well as to the activities of water and oxygen. They should therefore preserve important information about the conditions of crystallization or metamorphism of rocks throughout much of the Earth's crust. The stability relations of epidote-group minerals, however, are so far insufficiently well known to use to the full the information they contain. Even the relative stabilities of the iron-free end-member polymorphs clinozoisite and ortho- zoisite (or zoisite) are quite uncertain, despite their common coexistence as iron-poor polymorphs in metamorphic rocks.

In a recent issue of *Nature* however, Jenkins, Newton and Goldsmith (*Nature* **304**, 622; 1983) have shown unambiguously that Fe-free zoisite is significantly more stable than Fe-free clinozoisite, by experimentally studying their relative stabilities in equilibrium with kyanite, anorthite and quartz at high pressure and temperature. Zoisite has been found to grow in mixtures of zoisite and clinozoisite at temperatures as low as 350°C. Furthermore, Jenkins *et al.* have been able to suggest from a survey of occurrences of coexisting zoisite plus clinozoisite in metamorphic rocks that clinozoisite is only likely to be stable relative to zoisite below about 200°C. This elucidation of the relative stabilities of orthorhombic and monoclinic Fe-free epidote polymorphs constitutes an

important piece in the epidote jigsaw.

The surprisingly limited stability of monoclinic iron-free epidote (clinozoisite) indicated by Jenkins *et al.*'s work prompts speculation as to why monoclinic epidotes should be so widespread and common in crustal rocks. A quantitative solution to this problem is an important experimental and mineralogical task, which must focus on the substitution of iron for aluminium in the epidote structure. It is clear that the easy replacement of Al by Fe in the monoclinic epidotes relative to the ortho- zoisites enormously enhances their stability. But the inferred existence of a region of immiscibility in the clinozoisite-epidote solid solution underscores the likely mineralogical complexity of this solid solution. Jenkins *et al.*'s successful high-pressure study of a metastable mineral reaction involving clinozoisite, albeit over a narrow temperature interval between that of spontaneous nucleation and growth of zoisite at high temperature and that of prohibitively slow reaction at low temperature, paves the way for further systematic experimental study of the stabilities of the Fe-bearing monoclinic epidotes. Such a study, although experimentally complex, holds the prospect of locating more key pieces in the epidote jigsaw, and of allowing petrologists and mineralogists to make fuller use of epidote-group minerals than has been possible to date in deciphering the conditions of formation of a wide range of crustal rocks. □

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# Lead toxicity from water

**SIR** — In your discussion<sup>1</sup> of the merits of controlling various sources of lead, you suggest that greater importance should be given to the control of lead in dust. No method of dust control is specified, nor can we think of any feasible method. Our evidence suggests that the control of water lead by water hardening could be of greater general benefit than the control of dust.

Table 1 shows data for representative samples of children aged 1–3 years with their mothers, living in an area of very high soil-lead contamination from old lead mining, alongside a major road, in a *cul de sac* and in a small village. The subjects may be ordered according to the levels of house-dust lead to show the relative importance of dust in the blood lead of children and parents. The difference between the house-lead levels of dwellings on major roads and those in *culs de sac* is only 13 per cent. If dust on children's hands, estimated by a "wet wipe" technique<sup>2,3</sup> is also considered, there is no consistent pattern between children living alongside a major road and those in *culs de sac* or a village. Only in the area of exceptionally high lead contamination is there any large and significant correlation between dust and the lead on children's hands. On dietary lead, as it is

**Table 1** Lead levels in areas with differing dust lead levels

| Area              | n  | a   | b    | c    | d    |
|-------------------|----|-----|------|------|------|
| High soil lead    | 61 | 348 | 20.4 | 1.09 | 0.57 |
| Major road        | 42 | 200 | 15.1 | 0.80 | 0.43 |
| <i>Cul de sac</i> | 30 | 177 | 12.9 | 0.71 | 0.43 |
| Village           | 32 | 177 | 14.1 | 0.85 | 0.38 |

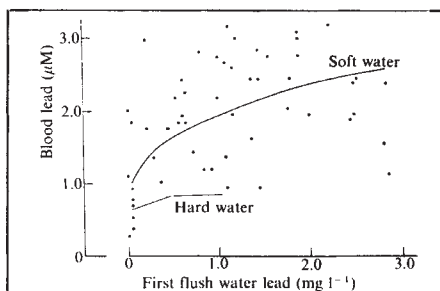
Key: a, Household dust Pb ( $\mu\text{g per g}$ ); b, children's hand Pb ( $\mu\text{g per g}$ ); c, children's blood Pb ( $\mu\text{M}$ ); d, maternal blood Pb ( $\mu\text{M}$ ).

**Table 2** Mean milk consumption and blood lead levels in women and children and the correlation coefficient (r) between them

|                                  | Women<br>(n = 175) | Children<br>(n = 39) |
|----------------------------------|--------------------|----------------------|
| Milk consumption<br>(ml per day) | 346 $\pm$ 222      | 625 $\pm$ 358        |
| Blood lead ( $\mu\text{M}$ )     | 0.60 $\pm$ 0.32    | 0.48 $\pm$ 0.19      |
| Correlation<br>coefficient       | 0.09               | -0.08                |

$\pm$  Indicates standard deviation.

known that calcium interferes with the absorption of lead<sup>4</sup>, we have examined the relationship with milk as a potential inhibitor of lead uptake. Table 2 shows data for children under 14 years and their mothers in a village in North Wales. No support for the hypothesis that milk consumption affects blood lead was found. There are several possible explanations<sup>5,6</sup> but the dietary inhibition of lead absorption by milk is certainly not a simple matter and the control of dietary lead could be accomplished only by more expensive controls on processing and packaging or by the addition to food of lead absorption in-



**Fig. 1** The relationship of blood and water lead in women in a soft water area and in a hard water area.

hibitors, neither of which has much appeal.

On water lead, several studies suggest a curvilinear relationship between water lead and blood lead<sup>7–9</sup>. Figure 1 shows data for two random samples of women, one sample from a hard water area ( $n=83$ ), the other from a soft water area ( $n=106$ ). In addition to indicating a difference in blood lead in the presence of raised water lead levels, these data suggest a difference in subjects in dwellings with negligible water lead. This suggests that hard water may reduce the absorption of lead from sources other than water, such as food.

These conclusions have been supported by an intervention study<sup>10</sup>. A random sample of women stratified by water lead level

in a plumbosolvent area showed a reduction of blood lead with hardening of water. A reduction in blood lead was shown even where initial water lead levels were negligible; no reduction was shown in a nearby area where water was not hardened.

In general, the highest blood lead levels seem to occur in soft water areas<sup>11</sup>, so our data suggest that the hardening of soft water supplies could make a substantial contribution to the general reduction of blood lead in this country.

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## Pyrex, quartz and "polywater"

**SIR** — Professor B. Derjaguin states<sup>1</sup> that certain samples of polywater "were products of leaching of quartz in the water condensate. This is the most reliable confirmation of the need to explain why the dissolving power of water formed by condensation of vapour is many times greater than that of liquid water at the same temperature" (emphasis added). The statement in italics would be important, if correct, because it proposes differential solvent properties of water, as a function of elapsed time since condensation. If this were the case, all capillaries in a Derjaguin experiment would contain "polywater". In fact "polywater" columns are found in only about 10 per cent of quartz capillaries occupying only about 20 per cent of the interior volume<sup>2</sup>.

I have proposed<sup>2</sup>, an alternative interpretation of the phenomena in Pyrex and quartz capillaries. My interpretation was that Pyrex contains hygroscopic alkaline compounds partly localized in microregions of the inner surface of the capillary tubes in which "polywater" is produced. These microregions preferentially attract water molecules from the vapour phase, forming microdroplets of highly concentrated alkaline solution which attack the adjacent Pyrex surfaces, forming a hygroscopic silicate solution. Droplets of the latter attract more water molecules from the vapour phase, until a

visible liquid column develops. This liquid has the composition and properties of a sodium silicate solution, in accordance with the analytical determinations. The effect is not observed when the capillaries are filled with liquid water as the alkaline solution is too dilute.

A similar sequence of events may occur in purified quartz capillaries if the extremely low proportion of impurities is represented by alkaline compounds segregated in microregions. In addition, there is the possibility<sup>2</sup> that regions of "Silica W" are formed when fused quartz is heated to about 1,665°C, during the drawing of capillary tubes. Silica W reacts rapidly with water vapour to form amorphous silicic acid and with liquid water to form unstable metasilicic acid which disperses into the liquid phase. This interpretation is also in accord with the consistent observation that columns of polywater do not form in all capillaries, do not fill the capillaries in which they do form, and form in a higher proportion of Pyrex capillaries than quartz capillaries. Thus there is no anomaly to be explained and no need to invoke special solvent properties of freshly condensed water.

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# The young Sun and the atmosphere and photochemistry of the early Earth

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*The origin and evolution of the Earth's early atmosphere depend crucially on the dissipation time  $t_N$  of the primitive solar nebula, SN. Using different theories of turbulence, we estimate that for a  $0.1 M_\odot$  SN,  $t_N$  is 2.5–8.3 Myr. Because accretion times are usually much longer, we conclude that most planetary accretion must have occurred in a gas-free environment. Using new IUE data, a wavelength-dependent UV flux is constructed for the young Sun which is then used to study the photochemistry and concentrations of O, O<sub>2</sub>, O<sub>3</sub>, OH, H, HCO and formaldehyde H<sub>2</sub>CO in the Earth's early prebiological atmosphere.*

THERE is convincing observational evidence that the placental interstellar medium (ISM) from which the Solar System originated was a dense molecular cloud<sup>1,2</sup>. In fact, the recent evidence of the presence of short-lived nuclei in meteorites<sup>2</sup> requires that the free-fall time scale for gravitational collapse ( $t_H$ ) be less than or comparable with the mean lifetime of <sup>26</sup>Al ( $10^6$  yr), that is  $t_H \leq 4 \times 10^7 / \sqrt{n_H} < 10^6$  yr, which requires<sup>2</sup>  $n_H > 10^3 \text{ cm}^{-3}$ , a value typical of molecular clouds. As molecular clouds are observed to be a major feature in our Galaxy, they constitute a reliable starting point for the processes that will eventually lead to the formation of stars and planetary systems<sup>3</sup>. A detailed analysis of the events that take place in such clouds, after an external agent has caused them to become unstable, has been worked out for a large variety of initial conditions<sup>4,5</sup>. Typically:  $M/M_\odot = 10^4$ ,  $\rho = 1.7 \times 10^{-23} \text{ g cm}^{-3}$ ,  $T = 75 \text{ K}$ ,  $R = 6.6 \times 10^{19} \text{ cm}$ ,  $\Omega = 10^{-15} \text{ rad s}^{-1}$ , and  $J/M = 1.75 \times 10^{24} \text{ cm}^2 \text{ s}^{-1}$ . Since T-Tauri stars have  $J/M = 10^{17} - 10^{18} \text{ cm}^2 \text{ s}^{-1}$ , a major effort has been dedicated to understand how to achieve a reduction of about 6 orders of magnitude in the value of  $J/M$ . The analysis indicates that the  $J/M$  reduction may be best achieved through a hierarchy of collapse and fragmentation and that the process yields binary fragments, a reassuring result in view of the preponderance of binary star systems. The most relevant result of these computations is that the fragments form with masses larger than the local Jeans masses, ensuring their continuous collapse, thus placing, the hierarchical fragmentation model on firm foundations<sup>6</sup>.

Recent work<sup>6,7</sup> has, however, indicated difficulties with the previous scenario. Study of the collapse of a dark cloud ( $M = 50 M_\odot$ ,  $T = 10 \text{ K}$ ,  $R = 2 \times 10^{18} \text{ cm}$ ,  $\Omega = 3 \times 10^{-14} \text{ rad s}^{-1}$ ,  $J/M = 4 \times 10^{22} \text{ cm}^2 \text{ s}^{-1}$ ) indicates that a binary system is first formed with  $M/M_\odot = 8$ , each of the two fragment dividing further into a triple system with  $M/M_\odot = 2$ ,  $R = 2 \times 10^{15} \text{ cm}$ ,  $J/M = 10^{20} \text{ cm}^2 \text{ s}^{-1}$ . The dynamics of each triple system indicates<sup>8</sup> that one object will be ejected in a time scale of  $t \approx 10^5$  yr, thus isolating one of the three partners which may then be considered a candidate for a massive primitive solar nebula as demanded in ref. 9. This sequence of events may, however, not be the true endpoint of the fragmentation process. A recent study<sup>7</sup> of the fate of the  $2 M_\odot$  ejected body indicates that it will further fragment into a multiple system, a process that will stop only at  $M < 0.1 M_\odot$ . These results show that fragmentation is not a self-regulating mechanism until one reaches  $M \sim 10^{-2} M_\odot$ , in agreement with an earlier analytic result<sup>10</sup> on opacity limited fragmentation. It has been suggested<sup>11</sup> that turbulence rather than simple fragmentation may be the relevant mechanism at work in star formation. If so, the resulting mass interval is  $5 \times 10^{-2} \leq M/M_\odot \leq 1$ , thus suggesting a solar nebula less massive than that of ref. 9 (see also ref. 12).

## Protostellar evolution—UV spectrum

Observationally, the pre-main-sequence stars appear to pass through three phases of evolution. While extremely young, the protostars are detected primarily at IR wavelengths. This phase is thought to correspond with the accretion of the protostellar core, obscured beyond a shroud of dust. Theoretical models<sup>13</sup> indicate that a  $1 M_\odot$  star will remain in this early stage for  $10^5$  yr.

The second phase, characterized by the T-Tauri stars, begins as the protostars become visible. By this time, relatively small amount of gas and dust accompany the star; the stellar photosphere and bright chromosphere may be seen. The termination of this phase may be estimated from the maximum observed ages of the stars. Examinations of several associations, using member hot stars of known ages and stability arguments, indicate ages of 3–5 Myr (refs 14, 15). If the T-Tauri stage corresponds to the fully convective phase of pre-main-sequence evolution (vertical portion of the tracks of Fig. 1), then theoretical predictions may be used<sup>16</sup>: 2 Myr for a  $2 M_\odot$  star, 12 Myr for a  $1 M_\odot$  star, and 40 Myr for a  $0.7 M_\odot$  star. The ages for several T-Tauri stars in several young associations and clusters have been estimated from their HR diagram<sup>17</sup>. Figure 1 shows three evolutionary tracks, superimposed on data obtained for stars in Taurus-Auriga and Orion<sup>17</sup>, indicating ages from  $10^5$  to  $10^7$  yr. Considering that the stars are variable, rotate, possess magnetic fields and lose mass, complications with which the theory cannot yet deal completely, the overall agreement among ages derived by all these methods is good. Thus we conclude that, for a solar mass star, the T-Tauri stage of pre-main-sequence evolution begins when the star is  $\sim 10^5$  yr old and lasts until it is  $\sim 6$ –12 Myr old.

The phase following the T-Tauri stars, the radiative phase, although it comprises over 80% of the time required for the star to evolve to the main sequence, is not as well-defined observationally. Herbig<sup>18</sup> postulated the probable characteristics of the post T-Tauri star. Unexpectedly, a few such stars have been found through X-ray detection by the Einstein satellite<sup>19,20</sup>. The post T-Tauri stars exhibit the characteristics expected for protostars which are more evolved than the T-Tauri stars. However, no clear difference in ages has yet been established<sup>21</sup>. Thus clear examples of solar mass protostars with ages of 10–50 Myr are as yet lacking.

The Sun is a relatively normal star of moderate mass, temperature and luminosity; it is very reasonable to assume that it passed through these three phases of pre-main-sequence evolution. Therefore, we can use UV observations of pre-main-sequence stars to represent the UV spectrum of the youthful Sun. Several such stars have been observed with the International Ultraviolet Explorer (IUE)<sup>22–26</sup>. Virtually all of the data

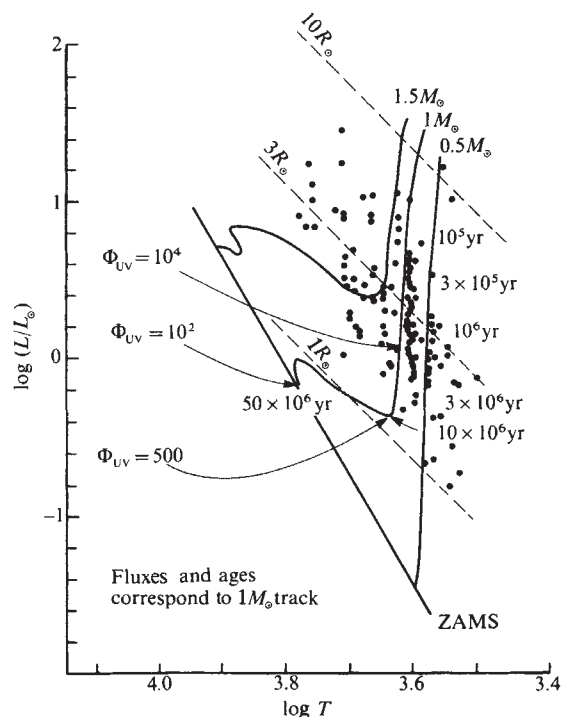
have been obtained for the brighter T-Tauri stars. Thus we use IUE spectra of three of the best studied T-Tauri stars to represent the probable solar UV spectrum at roughly an age of 1–10 Myr. We have drawn on comprehensive data (acquired by two of us) for three bright T-Tauri stars, SU Aurigae, RW Aurigae, and T Tauri (prototype of the class). The three stars form a reasonable sample of the heterogeneous class, representing a range of T-Tauri characteristics. Fourteen low-resolution IUE spectra of the three stars were averaged to produce a composite T-Tauri UV spectrum. The composite spectrum lacks information at the short wavelength end concerning the very strong hydrogen Ly $\alpha$  line because of geocoronal Ly $\alpha$  emission contamination. A weak stellar Ly $\alpha$  emission feature can be seen in the spectrum of RW Aurigae, superimposed on the geocoronal feature. A rough estimate of the flux indicates that the Ly $\alpha$  line in RW Aur is about 1/10 as strong as its hydrogen Balmer- $\alpha$  emission line<sup>27</sup>. Thus we have estimated the Ly $\alpha$  fluxes for the three T-Tauri stars as 0.1 times their respective Balmer- $\alpha$  fluxes. These estimates are consistent with the non-detections in SU Aur and T Tau. To complete the composite T-Tauri spectrum we have used published visual fluxes of these stars<sup>27</sup>. Small gaps in the UV and visual data were interpolated, as no strong emission lines are expected at those wavelengths. The mean spectrum of the visual spectra of the three stars was formed and reduced to the flux intercepted at a distance of 1 AU. A conservative correction for dust extinction corresponding to  $A_v = 0.5$  mag was made. The resulting T-Tauri spectrum normalized to the present solar value is presented in Fig. 2. Three strong emission lines are notable: Ly $\alpha$  (121 nm), Mg II (280 nm) and Balmer  $\alpha$  (656 nm). The greater luminosity of the average T-Tauri star may be seen at all wavelengths. However, the ratio is a strong function of wavelength: at the shortest wavelengths to which the photochemical processes are most sensitive, a young star is brighter than the present Sun by  $>10^4$ .

Thus, the use of this observationally determined UV spectrum rather than a wavelength-independent approximation<sup>28</sup> constitutes a significant improvement to the input for modelling the photochemistry of the early atmosphere.

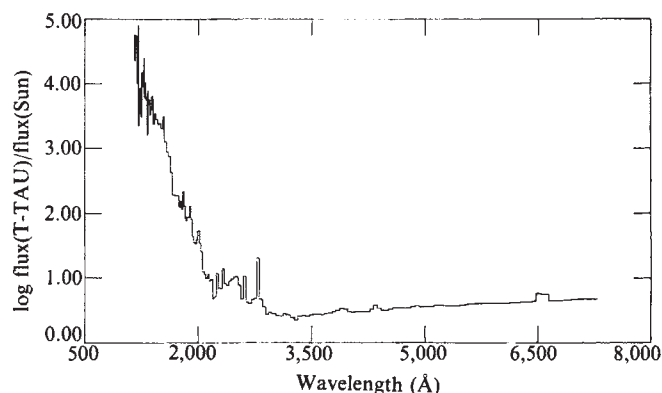
It might be argued that the extreme level of UV emission seen in the T-Tauri stars does not characterize the solar UV spectrum of the Sun's subsequent evolution towards the main sequence. The lack of known 'old' post T-Tauri stars does not allow us a direct observational test. However, Bøsegaard and Simon<sup>29</sup> have studied the changes in the UV emission lines originating in the stellar chromosphere (corresponding to  $\lambda < 1,900$  Å) with age for several young main sequence stars. The line fluxes, normalized to stellar surface areas, fall off roughly as  $t^{-1/2}$ . We have used this result to estimate how the emission line flux decreases with time after the T-Tauri phase<sup>28</sup>. We find that, even after the Sun has reached the main sequence, its far-UV luminosity is still  $10^2$  times the present value. The slow decrease in the UV flux means that the results derived here for the T-Tauri phase are largely applicable, with moderate down-scaling, to the later stages of the Sun's pre-main-sequence evolution.

## Earth formation—solar nebula dissipation

The accretion process that led to the formation of the earth from grains, dust and gas has been studied by several authors<sup>12,30–36</sup>. (For a detailed review, see ref. 32.) While early estimates by Schmidt<sup>30</sup> resulted in an accretion time  $t_A \approx 250$  Myr, all recent analyses<sup>32</sup> yield considerably lower values, that is  $23 \leq t_A \leq 88$  Myr. These results assume that accretion occurred in the absence of gas<sup>32</sup>. In the presence of gas<sup>32–35</sup>,  $t_A$  decreases to  $\sim 10$  Myr, and a massive atmosphere ( $\sim 10^4$  times the present one or the one corresponding to the gas-free situation) results. Since the two models lead to very different conclusions for the Earth's atmosphere, it is important to analyse their basic ingredients. A critical role is played by  $t_N$ , the dissipation time of the solar nebula, whose value depends in most models considered thus far, on the turbulent viscosity



**Fig. 1** The pre-main-sequence evolution of  $0.5M_{\odot}$ ,  $1M_{\odot}$  and  $1.5M_{\odot}$  stars. ●, Observational data<sup>17</sup>. The increase in the UV flux  $\Phi_{UV}$  (measured in terms of its present value) as well as the ages for  $1M_{\odot}$  star have been indicated. It is seen that at  $t = 10^7$  yr, corresponding to the end of the T-Tauri phase, the UV flux was 500 times stronger than today.



**Fig. 2** The composite UV flux (normalized to its present value) as derived using 14 spectra of three T-Tauri stars. In the region of importance to photochemical processes, the UV flux is  $10^4$  greater than its present value.

$v_t = \xi l v_t$  (where  $l$  and  $v_t$  are typical lengths and velocities of the turbulent motion). A detailed analysis<sup>37</sup> leads to the following dependence of  $t_N$  on  $\xi$

$$t_N = \frac{A}{4\xi} \text{ Myr} \quad (1)$$

where  $A = (M_N/10^{-1}M_{\odot})^{1.37} (X/50 \text{ AU})^{-1.37} (V_w \dot{M}_w/2 \times 10^{26})^{-1.33}$ . Here,  $M_N$  is the mass of the solar nebula,  $X$  its extension,  $V_w$  the solar wind and  $\dot{M}_w$  the rate of mass loss<sup>38–41</sup>. In the absence of a direct estimate of  $\xi$ , one can only say that<sup>33</sup>

$$10^{-3} \leq \xi \leq 1/3 \quad (2)$$

a result arrived at as follows. In the standard treatment of a turbulent fluid, one envisages three regions characterized by different sizes of the eddies there contained. In the first region, the eddy sizes  $l$  are comparable with  $L$ , the size of the system itself. In this case one writes  $\nu = \xi_L \nu_l$ , where  $\xi_L$  is the inverse of the Reynolds number  $Re$  which, for turbulence to set in, must be  $>10^3$ , and so  $\xi_L < 10^{-3}$ , yielding the lower limit in



equation (2). At the other end of the spectrum, the eddies are sufficiently small for molecular forces to dominate, and so  $\nu = \xi_m \nu_l$ , where  $\xi_m = 1/3$ , thus yielding the upper unit in equation (2).

In the intermediate size region, containing all the turbulent phenomena,  $\nu_i = \xi l_i \nu_l$  and  $\xi$  must be computed from first principles. Using the Heisenberg–Weizsacker's theory of turbulence<sup>42</sup>, one has

$$\nu_i(k) = \gamma \int_k^\infty \left( \frac{F(k)}{k^3} \right)^{1/2} dk, \quad (3)$$

$$\nu_i^2 = \int_k^\infty F(k) dk, \quad l_i = \pi/k$$

where  $F(k)$  is the spectral-energy distribution function solution of the dynamical equations governing turbulence. We have computed  $\xi$  using:

(1) Kolmogoroff spectrum<sup>42</sup>,  $F(k) \sim k^{-5/3}$ . In this case

$$\xi = \frac{\gamma}{\pi} \sqrt{\frac{3}{8}} = 0.06, \quad \gamma = 1/3 \quad (4)$$

(2) The Steward–Townsend<sup>42</sup> experimental spectrum  $F(k) \sim k^{-\alpha \ln k}$ . We obtain

$$\xi = \frac{\gamma q}{\pi} \sqrt{\frac{2}{\alpha}} \frac{1 - \Phi(x)}{\{1 - \Phi(y)\}^{1/2}} \quad (5)$$

where  $x\sqrt{2} = \alpha \ln q + 0.5\alpha^{-1}$ ,  $y = \alpha \ln q - 0.5\alpha^{-1}$ ,  $q = k/k_0 = L/l$ . We solved equation (5) numerically. For a wide range of parameters, the resulting values of  $\xi$  are

$$0.03 \leq \xi < 0.08 \quad (6)$$

(3) The theory of convective turbulence developed in ref. 43 and valid for  $R\sigma \ll 1$ , where  $R$  is the Rayleigh number and  $\sigma$  the Prandtl number. Since  $F(k)$  cannot be given in closed analytical form, the evaluation of  $\xi$  was done numerically. For the range  $10^{-7} < \delta < 10^{-1}$  of  $\delta = 8\pi^4/R$ , the result is

$$0.03 < \xi < 0.07 \quad (7a)$$

(4) We have extended the theory of ref. 43 to the case  $R\sigma \gg 1$ . Because the entire analysis must be done numerically, we report only the final result, that is

$$0.06 \leq \xi \leq 0.07 \quad (7b)$$

(5) The theory of convective turbulence developed by one of us (V.M.C., in collaboration with I. Goldman) in which the Heisenberg expression (3) for  $\nu_i$  is changed to the integral over  $k$  of the ratio  $F(k)/n(k)$ , where  $n(k)$  is the growth rate of the laminar modes<sup>43</sup>. The result is ( $H^2 = h/2\lambda q^2$ ,  $h = (1 + \lambda^2 q^4)^{1/2} - \lambda q^2$ ,  $C_\lambda^2 = (1 + \lambda^2)^{1/2} + \lambda$ )

$$\xi = \left( \frac{\gamma_* C_\lambda}{\pi^2} \right)^{1/2} q (2\lambda)^{1/4} \frac{(1 + H^2)^{1/2} - 1}{(H - \lg^{-1} H)^{1/2}} \quad (8)$$

where  $\lambda^2 = 2\pi^4/R\sigma$  and  $\gamma_* = 2/15$  (ref. 44). For  $\lambda \gg 1$

$$\xi = \left( \frac{3\gamma_*}{4\pi^2} \right)^{1/2} = 0.1 \quad (9)$$

Using these results, as well as that of ref. 44, i.e.  $\xi = 0.05$ , we conclude that for  $A = 1$

$$t_N = 2.5\text{--}8.3 \text{ Myr} \quad (10)$$

A similar result,  $t_N \sim 10$  Myr, has also been obtained in ref. 45. Note that the upper limit of  $t_N$  closely corresponds to the point where the T-Tauri phase (vertical part of the  $1M_\odot$  track, Fig. 1) ends. Furthermore, because either value of  $t_N$  is smaller than the values<sup>32</sup> of  $t_A$ , it seems unlikely that gas might have influenced accretion to the point of producing a massive  $H_2$  atmosphere. Because the quoted values of  $t_A$ , ranging from 10 Myr to 23–88 Myr correspond to extreme assumptions as far as the gas is concerned, our result suggesting a gradual dispersion of the gas while the Earth was accreting, would in principle require a new evaluation of  $t_A$  with a time-decreasing gas density. However, for the present purposes, it is sufficient to take an average of the previous values. If we take  $t_A = 50$  Myr, we would conclude that the SN gas was present only for 10% of  $t_A$ , and that most of the accretion occurred while the Sun was moving

along the radiative track stage of Fig. 1. When the Earth was accreted, the UV flux was therefore about  $10^2$  times larger than its present value, large enough to influence the photochemistry of its atmosphere significantly.

## Origin of the atmosphere

Having concluded that the SN was an unlikely contributor to the Earth's atmosphere, one may consider the following potential contributors<sup>46</sup>: (1) collision with comets<sup>47</sup>, (2) volatilization of impacting material and (3) outgassing. One may distinguish two periods of outgassing<sup>48</sup>. While the accretion energy due to impact was in principle sufficient to melt the entire Earth, this may not have occurred, since a fully melted Earth would have expelled all volatiles, whilst it is known that  $^3\text{He}$  is still degassed today. Accretional heating may have produced partial melting including Fe alloys that migrated towards the centre forming ultimately the Earth's core. The time scales for the core formation discussed in the past ( $\approx 10^9$  yr) have been shown to be too long. The conclusion is that the core formed contemporaneously with the accretion of the Earth itself, that is in  $\approx 10^7$  yr (ref. 48). During this period, two sources of volatiles therefore acted simultaneously, the outgassing caused by the impacting material that stirred the mantle and the impacting meteorites. Because volatiles are bound in these meteorites, the energy retained as heat on impact would be sufficient to vaporize the volatile species, releasing them so as to form an atmosphere. As stated in ref. 49 this conclusion seems unavoidable: the formation only of a solid body with the subsequent outgassing seems an artificial division of events which more naturally should be thought of as having occurred simultaneously. As the embryonic Earth continued to grow, the impact velocities increased until vaporization of the impacting material occurred. Non-volatile solids so vaporized cooled and condensed onto the surface. Volatiles such as  $H_2O$ ,  $CO_2$ ,  $N_2$ , and noble gases probably remained in the atmosphere. Calculations<sup>50</sup> indicate that this process occurs for impact velocities  $> 5 \text{ km s}^{-1}$ , which in the case of the Earth was achieved<sup>51</sup> when the radius was 50% of its present value, that is before  $\sim 10^6$  yr (Fig. 10 of ref. 52).

The chemical composition of this atmosphere depends critically on complex chemical reactions between the infalling vaporized gas, the outgassed volatiles and other elements, particularly Fe (G. W. Wetherill, personal communication). In the specific impact originated atmosphere studied in ref. 50, the partial pressure of water was estimated to be  $P_{H_2O} \approx 0.01$  bar. The fate of atmospheric  $CO_2$  is difficult to quantify and experiments by Ahrens and collaborators (D. J. Stevenson, personal communication) are expected to shed light on this point, because carbonate growth may have removed a great deal of  $CO_2$  during the time scale of the Earth's accretion. The first phase of outgassing, together with an impact-originated atmosphere, lasted a period of time not greatly different from the accretion time itself, at the end of which the Earth entered a period in which accretion is over, the mantle solid and the core fully formed<sup>48</sup>. Such a period is characterized by a large 'mass flux' which transports large masses of the mantle to the surface region, thus making them available for the second period of outgassing. Because the length of this period is difficult to estimate, the following argument can be made<sup>53</sup>. The abundance of  $^{129}\text{Xe}/^{130}\text{Xe}$  in the atmosphere is smaller than that in the solid Earth, the latter being due to enrichment caused by the decay of  $^{129}\text{I}$  to  $^{129}\text{Xe}$ . If the outgassing took much longer than a few lifetimes of  $^{129}\text{I}$  (17 Myr), at the end of it there would have been no  $^{129}\text{Xe}$  left and both the atmospheric and the terrestrial  $^{129}\text{Xe}$  should have been equally abundant. As this is not the case, the separation of the  $^{129}\text{Xe}$  from the Earth (outgassing), must have occurred while  $^{129}\text{I}$  was still present, that is within a few  $^{129}\text{I}$  lifetimes, say 50 Myr. (However, as Wetherill has noted, the argument is also consistent with no outgassing of the Earth at all, all the atmospheric  $^{129}\text{Xe}$  having perhaps been deposited in the atmosphere by impact.)

We therefore conclude that the entire set of phenomena, (1) collapse of the nebula, (2) accretion of the Earth, (3) dissipation

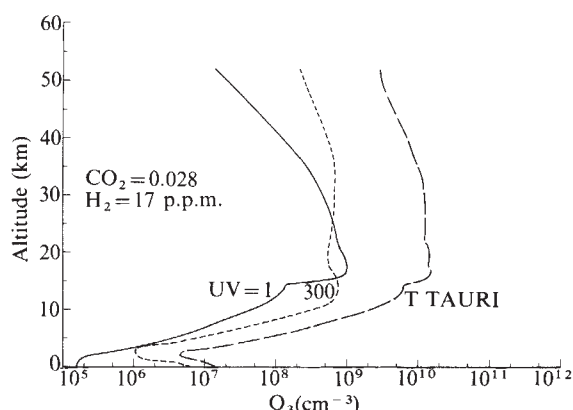
of the nebular remnant, (4) formation of the Earth's core, (5) formation of an atmosphere by impact volatiles plus outgassing and (6) contribution to the atmosphere by the second outgassing period, may have occurred within 50–100 Myr, corresponding to the time when the Sun entered the main sequence (Fig. 1).

On the basis of the previous discussion, for our photochemical calculations we have assumed a background composition of the palaeoatmosphere consistent with both the impact and outgassing scenarios. We adopt an  $\text{H}_2\text{O}$  vapour partial pressure of 0.01 bar, as from ref. 50. The  $\text{H}_2\text{O}$  mixing ratio varies from  $1.2 \times 10^{-2}$  at the surface to  $4.6 \times 10^{-7}$  at the tropopause. Above it, the distribution of  $\text{H}_2\text{O}$  vapour is calculated using a continuity/transport equation for  $\text{H}_2\text{O}$ . Because of the lack of firm experimental evidence concerning the rate of removal of atmospheric  $\text{CO}_2$  due to carbonate growth, the level of  $\text{CO}_2$  cannot be uniquely determined. We have therefore performed our photochemical calculations for two representative values:  $\text{CO}_2 = 1$  (the pre-industrial level of 280 p.p.m.v.) and  $\text{CO}_2 = 100$  times that value. Higher values of  $\text{CO}_2$  seem improbable<sup>45</sup>. Furthermore, we have adopted 0.8 bar of  $\text{N}_2$ , a chemically inert gas that participates in the chemical reactions only as a third

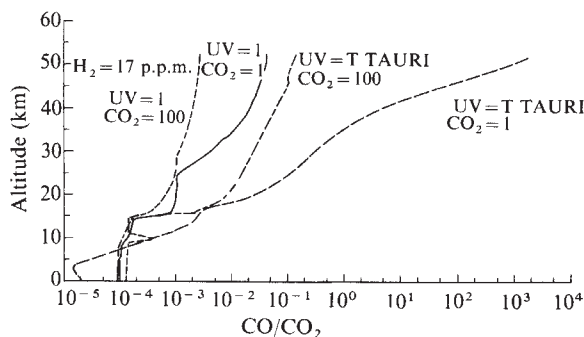
body. We have also added the trace gases  $\text{H}_2$  and  $\text{CO}$ . Calculations were performed for the two values of the surface mixing ratios  $\text{H}_2$ , namely 17 p.p.m.v. and  $10^{-3}$  (refs 54, 55). The surface flux of  $\text{CO}$  was taken to be  $2 \times 10^8$  (molecules  $\text{cm}^{-2} \text{s}^{-1}$ ) which corresponds to a surface mixing ratio of parts per billion by volume.  $\text{CH}_4$  and  $\text{NH}_3$  were not included for reasons explained elsewhere<sup>56,57</sup>.

## Photochemistry of oxygen, ozone and formaldehyde

Studies dealing with the origin and evolution of life<sup>58</sup> have indicated that formaldehyde,  $\text{H}_2\text{CO}$ , was a key molecule in the process of chemical evolution, that is the formation of complex organic molecules. Today,  $\text{H}_2\text{CO}$  is a trace species produced by the methane oxidation chain with the precursor  $\text{CH}_4$  being provided by biogenic activity. Because the lifetime of  $\text{CH}_4$  in the prebiological atmosphere is extremely short<sup>56,57</sup> due to photochemical destruction, the question then arises as to how to produce  $\text{H}_2\text{CO}$  in the absence of a steady source of  $\text{CH}_4$ . A prebiological photochemical source for  $\text{H}_2\text{CO}$  has been suggested<sup>55</sup>. As we shall show below, it is sensitive to the UV flux



**Fig. 3** The ozone profile. The solid curve ( $UV = 1$ ) corresponds to the present UV flux. The curve labelled  $UV = 300$  corresponds to a wavelength-independent increase by a factor of 300. The curve labelled T Tauri corresponds to the use of the flux of Fig. 2. A substantial increase in the amount of  $\text{O}_3$  can be seen (see Tables 3, 4).



**Fig. 4** The vertical profile of the ratio  $\text{CO}/\text{CO}_2$  of interest to laboratory simulation experiments for the formation of organic molecules<sup>61</sup>. It can be seen that only with an enhanced UV flux does one obtain  $\text{CO}/\text{CO}_2 \geq 1$ .

**Table 1** Solar energy flux averaged over the Earth during T-Tauri phase (numbers in parentheses correspond to present day values)

| Source          | Energy<br>( $\text{cal cm}^{-2} \text{yr}^{-1}$ ) |
|-----------------|---------------------------------------------------|
| Total radiation | $4.6 \times 10^6$ ( $0.26 \times 10^6$ )          |
| UV light        |                                                   |
| < 3,000 Å       | $2.4 \times 10^4$ ( $0.34 \times 10^4$ )          |
| < 2,500 Å       | $9.9 \times 10^3$ ( $0.56 \times 10^3$ )          |
| < 2,000 Å       | $5.0 \times 10^3$ (40)                            |
| < 1,500 Å       | $2.2 \times 10^3$ (1.7)                           |

**Table 2** Photochemical and chemical reactions

| Reaction no. | Reaction                                                                        | Rate constant*<br>( $\text{s}^{-1}$ , $\text{cm}^3 \text{s}^{-1}$ or $\text{cm}^6 \text{s}^{-1}$ ) |                       |
|--------------|---------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------|-----------------------|
| J1           | $\text{O}_2 + h\nu \rightarrow \text{O} + \text{O}$                             | $4.7 \times 10^{-10}$                                                                              | $6.3 \times 10^{-9}$  |
| J2           | $\text{O}_3 + h\nu \rightarrow \text{O} + \text{O}_2$                           | $2.2 \times 10^{-4}$                                                                               | $8.5 \times 10^{-4}$  |
| J3           | $\text{O}_3 + h\nu \rightarrow \text{O}(^1\text{D}) + \text{O}_2$               | $4.4 \times 10^{-3}$                                                                               | $2.8 \times 10^{-2}$  |
| J4           | $\text{H}_2\text{O} + h\nu \rightarrow \text{OH} + \text{H}$                    | $1.1 \times 10^{-14}$                                                                              | $5.1 \times 10^{-13}$ |
| J5           | $\text{H}_2\text{O}_2 + h\nu \rightarrow \text{OH} + \text{OH}$                 | $5.3 \times 10^{-5}$                                                                               | $4.4 \times 10^{-4}$  |
| J6           | $\text{H}_2\text{CO} + h\nu \rightarrow \text{H} + \text{HCO}$                  | $4.8 \times 10^{-5}$                                                                               | $1.6 \times 10^{-4}$  |
| J7           | $\text{H}_2\text{CO} + h\nu \rightarrow \text{H}_2 + \text{CO}$                 | $4.9 \times 10^{-5}$                                                                               | $1.4 \times 10^{-4}$  |
| J8           | $\text{CO}_2 + h\nu \rightarrow \text{CO} + \text{O}$                           | $1.3 \times 10^{-12}$                                                                              | $6.3 \times 10^{-11}$ |
| J9           | $\text{HCO} + h\nu \rightarrow \text{H} + \text{CO}$                            | $1.3 \times 10^{-2}$                                                                               | $5.8 \times 10^{-2}$  |
| 1            | $\text{O} + \text{O}_2 + \text{M} \rightarrow \text{O}_3 + \text{M}$            | $1.1 \times 10^{-34}$                                                                              | exp (510/T)           |
| 2            | $\text{O} + \text{O}_3 \rightarrow 2\text{O}_2$                                 | $1.5 \times 10^{-11}$                                                                              | exp (-2,218/T)        |
| 3            | $\text{O}(^1\text{D}) + \text{O}_2 \rightarrow \text{O} + \text{O}_2$           | $3.2 \times 10^{-11}$                                                                              | exp (67/T)            |
| 4            | $\text{O}(^1\text{D}) + \text{N}_2 \rightarrow \text{O} + \text{N}_2$           | $2.0 \times 10^{-11}$                                                                              | exp (107/T)           |
| 5            | $\text{H}_2\text{O} + \text{O}(^1\text{D}) \rightarrow 2\text{OH}$              | $2.3 \times 10^{-10}$                                                                              |                       |
| 6            | $\text{H} + \text{O}_2 + \text{M} \rightarrow \text{HO}_2 + \text{M}$           | $2.1 \times 10^{-32}$                                                                              | exp (290/T)           |
| 7            | $\text{H} + \text{O}_3 \rightarrow \text{OH} + \text{O}_2$                      | $1.4 \times 10^{-10}$                                                                              | exp (-470/T)          |
| 8            | $\text{OH} + \text{O} \rightarrow \text{H} + \text{O}_2$                        | $4.0 \times 10^{-11}$                                                                              |                       |
| 9            | $\text{OH} + \text{O}_3 \rightarrow \text{HO}_2 + \text{O}_2$                   | $1.6 \times 10^{-12}$                                                                              | exp (-940/T)          |
| 10           | $\text{OH} + \text{OH} \rightarrow \text{H}_2\text{O} + \text{O}$               | $1.0 \times 10^{-12}$                                                                              | exp (-500/T)          |
| 11           | $\text{HO}_2 + \text{O} \rightarrow \text{OH} + \text{O}_2$                     | $3.5 \times 10^{-11}$                                                                              |                       |
| 12           | $\text{HO}_2 + \text{O}_3 \rightarrow \text{OH} + 2\text{O}_2$                  | $1.1 \times 10^{-14}$                                                                              | exp (-580/T)          |
| 13           | $\text{HO}_2 + \text{OH} \rightarrow \text{H}_2\text{O} + \text{O}_2$           | $4.0 \times 10^{-11}$                                                                              |                       |
| 14           | $\text{HO}_2 + \text{HO}_2 \rightarrow \text{H}_2\text{O}_2 + \text{O}_2$       | $2.5 \times 10^{-12}$                                                                              |                       |
| 15           | $\text{H}_2\text{O}_2 + \text{OH} \rightarrow \text{HO}_2 + \text{H}_2\text{O}$ | $1.0 \times 10^{-11}$                                                                              | exp (-750/T)          |
| 16           | $\text{O}(^1\text{D}) + \text{H}_2 \rightarrow \text{OH} + \text{H}$            | $9.9 \times 10^{-11}$                                                                              |                       |
| 17           | $\text{OH} + \text{OH} + \text{M} \rightarrow \text{H}_2\text{O}_2 + \text{M}$  | †                                                                                                  |                       |
| 18           | $\text{H}_2\text{O}_2 + \text{O} \rightarrow \text{OH} + \text{HO}_2$           | $2.8 \times 10^{-12}$                                                                              | exp (-2,125/T)        |
| 19           | $\text{H}_2 + \text{OH} \rightarrow \text{H}_2\text{O} + \text{H}$              | $1.2 \times 10^{-11}$                                                                              | exp (-2,200/T)        |
| 20           | $\text{H}_2\text{CO} + \text{OH} \rightarrow \text{HCO} + \text{H}_2\text{O}$   | $1.7 \times 10^{-11}$                                                                              | exp (-100/T)          |
| 21           | $\text{H}_2\text{CO} + \text{O} \rightarrow \text{OH} + \text{HCO}$             | $2.8 \times 10^{-11}$                                                                              | exp (-1,540/T)        |
| 22           | $\text{HCO} + \text{O}_2 \rightarrow \text{CO} + \text{HO}_2$                   | $5.0 \times 10^{-12}$                                                                              |                       |
| 23           | $\text{CO} + \text{OH} \rightarrow \text{H} + \text{CO}_2$                      | †                                                                                                  |                       |
| 24           | $\text{O} + \text{O} + \text{M} \rightarrow \text{O}_2 + \text{M}$              | $2.8 \times 10^{-34}$                                                                              | exp (710/T)           |
| 25           | $\text{H} + \text{H} + \text{M} \rightarrow \text{H}_2 + \text{M}$              | $8.3 \times 10^{-33}$                                                                              |                       |
| 26           | $\text{H}_2 + \text{O} \rightarrow \text{OH} + \text{H}$                        | $3.0 \times 10^{-14}$                                                                              | exp (-4,480/T)        |
| 27           | $\text{H} + \text{HO}_2 \rightarrow \text{O}_2 + \text{H}_2$                    | $4.7 \times 10^{-11}$                                                                              | ( $\times 0.29$ )     |
| 28           | $\text{H} + \text{HO}_2 \rightarrow \text{H}_2\text{O} + \text{O}$              | $4.7 \times 10^{-11}$                                                                              | ( $\times 0.02$ )     |
| 29           | $\text{H} + \text{HO}_2 \rightarrow \text{OH} + \text{OH}$                      | $4.7 \times 10^{-11}$                                                                              | ( $\times 0.69$ )     |
| 30           | $\text{H} + \text{CO} + \text{M} \rightarrow \text{HCO} + \text{M}$             | $2.0 \times 10^{-33}$                                                                              | exp (-850/T)          |
| 31           | $\text{H} + \text{HCO} \rightarrow \text{H}_2 + \text{CO}$                      | $3.0 \times 10^{-10}$                                                                              |                       |
| 32           | $\text{HCO} + \text{HCO} \rightarrow \text{H}_2\text{CO} + \text{CO}$           | $6.3 \times 10^{-11}$                                                                              |                       |
| 33           | $\text{OH} + \text{HCO} \rightarrow \text{H}_2\text{O} + \text{CO}$             | $5.0 \times 10^{-11}$                                                                              |                       |
| 34           | $\text{O} + \text{HCO} \rightarrow \text{H} + \text{CO}_2$                      | $1.0 \times 10^{-10}$                                                                              |                       |
| 35           | $\text{O} + \text{HCO} \rightarrow \text{OH} + \text{CO}$                       | $1.0 \times 10^{-10}$                                                                              |                       |
| 36           | $\text{HO}_2 + \text{HCO} \rightarrow \text{H}_2\text{O}_2 + \text{CO}$         | $1.0 \times 10^{-11}$                                                                              |                       |
| 37           | $\text{H}_2\text{CO} + \text{H} \rightarrow \text{H}_2 + \text{HCO}$            | $2.8 \times 10^{-11}$                                                                              | exp (-1,540/T)        |
| 38           | $\text{H}_2\text{CO}$ and $\text{H}_2\text{O}_2$ rainout                        | $1.0 \times 10^{-6} \text{s}^{-1}$                                                                 |                       |

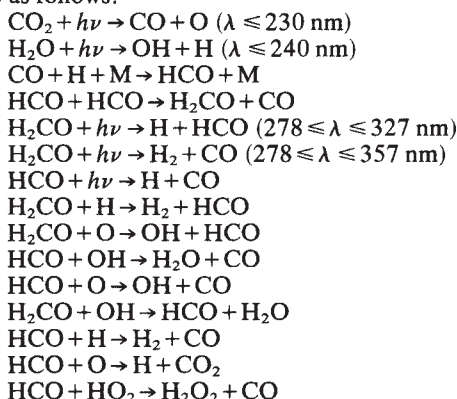
\* Photolysis rates ( $\text{s}^{-1}$ ) for  $\text{H}_2 = 17$  p.p.m.v. are given for  $UV = 1$  (first value) and  $UV = \text{T-Tauri phase}$ .

† See JPL Publ. 82-57 (1982).



and to the abundances of  $\text{CO}_2$  and  $\text{H}_2$ . The considerable increase of UV radiation suggested by the IUE data on young Sun may therefore alter the production rate of  $\text{H}_2\text{CO}$  estimated earlier<sup>55</sup>, a value of great interest because sufficient for polymerization purposes.

The production and destruction reactions suggested in ref. 55 are as follows:



These reactions were added to the one-dimensional photochemical model described in ref. 57 and used in ref. 28. The scheme was modified by eliminating the nitrogen and chlorine species, after it was found that they were irrelevant to the  $\text{H}_2\text{CO}$  photochemistry. The present version of the model contains 10 photochemical processes and 37 chemical reactions listed in Table 2. By simultaneously solving the coupled species continuity/transport equations, the surface concentrations and vertical profiles (up to 53.5 km) of the following species were computed: O,  $\text{O}_2$ ,  $\text{O}_3$ , OH, H, HCO,  $\text{H}_2\text{CO}$ , CO,  $\text{CO}_2$ ,  $\text{H}_2\text{O}$ ,

**Table 3** Surface concentration of several species ( $\text{cm}^{-3}$ ) as a function of UV flux and  $\text{CO}_2$  and  $\text{H}_2$  level

| Species                                                 | UV = 1            |                     | UV = T-Tauri phase |                     |
|---------------------------------------------------------|-------------------|---------------------|--------------------|---------------------|
|                                                         | $\text{CO}_2 = 1$ | $\text{CO}_2 = 100$ | $\text{CO}_2 = 1$  | $\text{CO}_2 = 100$ |
| <b><math>\text{H}_2 = 17 \text{ p.p.m.}</math></b>      |                   |                     |                    |                     |
| O                                                       | 8.95 (6)          | 4.47 (7)            | 4.92 (8)           | 1.96 (9)            |
| $\text{O}_2$                                            | 2.72 (5)          | 1.36 (9)            | 2.33 (7)           | 1.29 (10)           |
| $\text{O}_3$                                            | 5.66              | 1.69 (5)            | 2.20 (3)           | 1.46 (7)            |
| OH                                                      | 4.13 (3)          | 1.51 (4)            | 2.28 (5)           | 6.66 (5)            |
| CO                                                      | 5.09 (11)         | 7.86 (13)           | 1.10 (11)          | 5.16 (13)           |
| H                                                       | 3.24 (7)          | 4.09 (6)            | 1.36 (9)           | 1.28 (8)            |
| HCO                                                     | 1.39 (6)          | 2.06 (7)            | 5.58 (5)           | 2.50 (7)            |
| $\text{H}_2\text{CO}$                                   | 1.62 (6)          | 2.65 (8)            | 3.38 (4)           | 7.17 (7)            |
| $\text{CO}/\text{CO}_2$                                 | 9.02 (-5)         | 1.39 (-4)           | 1.95 (-5)          | 9.15 (-5)           |
| <b><math>\text{H}_2 = 10^{-3} \text{ p.p.m.}</math></b> |                   |                     |                    |                     |
| O                                                       | 2.10 (5)          | 6.57 (6)            | 9.49 (6)           | 1.44 (8)            |
| $\text{O}_2$                                            | 1.87 (2)          | 1.20 (4)            | 6.12 (4)           | 1.99 (6)            |
| $\text{O}_3$                                            | 1.07 (-4)         | 2.21 (-1)           | 2.18 (-1)          | 1.15 (2)            |
| OH                                                      | 7.64 (1)          | 2.60 (2)            | 3.67 (3)           | 1.45 (4)            |
| CO                                                      | 3.09 (13)         | 3.63 (15)           | 2.66 (13)          | 2.37 (15)           |
| H                                                       | 3.46 (6)          | 2.32 (5)            | 4.34 (7)           | 8.60 (6)            |
| HCO                                                     | 1.50 (7)          | 7.95 (7)            | 3.26 (7)           | 3.55 (8)            |
| $\text{H}_2\text{CO}$                                   | 4.14 (8)          | 4.12 (9)            | 3.79 (8)           | 2.43 (10)           |
| $\text{CO}/\text{CO}_2$                                 | 5.48 (-3)         | 6.44 (-3)           | 4.72 (-3)          | 4.20 (-3)           |

**Table 4** Column densities of  $\text{O}_3$  and  $\text{H}_2\text{CO}$  ( $\text{molecules cm}^{-2}$ )

|                                         | UV = 1            |                     | UV = T-Tauri phase |                     |
|-----------------------------------------|-------------------|---------------------|--------------------|---------------------|
|                                         | $\text{CO}_2 = 1$ | $\text{CO}_2 = 100$ | $\text{CO}_2 = 1$  | $\text{CO}_2 = 100$ |
| <b><math>\text{O}_3</math></b>          |                   |                     |                    |                     |
| $\text{H}_2 = 17 \text{ p.p.m.v.}$      | 3.3 (12)          | 1.5 (15)            | 7.4 (15)           | 4.05 (16)           |
| $\text{H}_2 = 10^{-3}$                  | 2.1 (11)          | 1.3 (14)            | 6.5 (14)           | 2.01 (15)           |
| <b><math>\text{H}_2\text{CO}</math></b> |                   |                     |                    |                     |
| $\text{H}_2 = 17 \text{ p.p.m.v.}$      | 5.4 (11)          | 7.34 (13)           | 5.9 (9)            | 5.45 (13)           |
| $\text{H}_2 = 10^{-3}$                  | 8.6 (14)          | 2.02 (15)           | 3.7 (14)           | 1.1 (16)            |

$\text{H}_2$ ,  $\text{HO}_2$  and  $\text{H}_2\text{O}_2$  using the UV flux of Fig. 2 to initiate photochemical processes. Water soluble species (such as  $\text{H}_2\text{CO}$  and  $\text{H}_2\text{O}_2$ ) are lost by rainout with a rainout coefficient of  $10^{-6} \text{ s}^{-1}$  (11.6 days), as derived from both theoretical calculations and direct measurements of the rainout lifetime of water soluble  $\text{NH}_3$  (ref. 59). Calculated surface concentrations of the species of interest are presented in Table 3. The column densities of  $\text{H}_2\text{CO}$  and  $\text{O}_3$  versus UV flux are presented in Table 4. Finally, the surface production, losses due to photochemistry/chemistry and rainout and the rainout rates for  $\text{H}_2\text{CO}$  are presented in Table 5.

## Results

The dissipation time scale of the SN presented here, equation (10), suggests that accretion probably occurred mostly in a gas-free environment. This, together with previous results, suggest that Earth accretion and the formation of an atmosphere (due to impact and first phase of outgassing), occurred within the interval of time it took the Sun to arrive at the main sequence. During this period, the UV flux was at least 100 times larger than its present value.

The pre-main-sequence solar UV flux was determined by using observational IUE data on the T-Tauri stars. Figure 2 shows the strong wavelength dependence of the increase in the UV flux. (Present solar fluxes were taken from ref. 60.) In Table 1, we present the solar energy flux during the T-Tauri phase as averaged over the Earth. While the total radiation is  $\sim 18$  times larger than its present value, the UV fluxes are increased up to  $10^4$ .

Our results show that the increment in the surface concentration and the vertical profile of  $\text{O}_2$  determined in ref. 28 are not changed. As we see from Table 3, the  $\text{O}_2$  mixing ratio increases up to  $10^4$ – $10^5$  times, depending on the value of  $\text{H}_2$ . Furthermore,  $\text{O}_2$  increases as the  $\text{CO}_2$  and UV fluxes increase, as expected.

While for  $\text{O}_2$  the quantity of interest is the surface concentration, for ozone the total column density is perhaps more relevant due to its ability to absorb UV radiation. Such a distribution turns out to be substantially altered by the use of a  $\lambda$ -dependent UV flux, compared with the  $\lambda$ -independent treatment of ref. 28. The results of Fig. 3 and Tables 3 and 4 indicate that there has been an increase in the ozone column density of 1–3 orders of magnitude.

Both O and OH radicals are seen to increase by an order of magnitude when either  $\text{CO}_2$  or the UV flux are increased.

The  $\text{CO}/\text{CO}_2$  ratio is a particularly interesting quantity in view of a recent laboratory experiment<sup>61</sup> indicating that a mixture of  $\text{H}_2\text{O}$  and CO, once irradiated with UV radiation, yields a large array of organic molecules relevant to the origin of life. In the absence of CO, irradiation of  $\text{CO}_2$  yields no organics. The profile of the  $\text{CO}/\text{CO}_2$  ratio is presented in Fig. 4 for  $\text{H}_2 = 17 \text{ p.p.m.}$ , corresponding to the minimum calculated ratio. It is seen that at high altitudes the ratio is still much smaller

**Table 5** Surface production and loss rates for  $\text{H}_2\text{CO}$  as a function of UV flux and  $\text{CO}_2$  level ( $\text{H}_2 = 17 \text{ p.p.m.v.}$ )

| Reaction                                                                      | Reaction rate ( $\text{molecules cm}^{-3} \text{ s}^{-1}$ ) |                     |                   |                     |
|-------------------------------------------------------------------------------|-------------------------------------------------------------|---------------------|-------------------|---------------------|
|                                                                               | UV = 1                                                      |                     | UV = T Tauri      |                     |
|                                                                               | $\text{CO}_2 = 1$                                           | $\text{CO}_2 = 100$ | $\text{CO}_2 = 1$ | $\text{CO}_2 = 100$ |
| 1. Production                                                                 |                                                             |                     |                   |                     |
| $\text{HCO} + \text{HCO} \rightarrow \text{H}_2\text{CO} + \text{CO}$         | 1.22 (2)                                                    | 2.67 (4)            | 1.97 (1)          | 3.93 (4)            |
| 2. Loss due to photochemistry/chemistry                                       |                                                             |                     |                   |                     |
| $\text{H}_2\text{CO} + h\nu \rightarrow \text{H} + \text{HCO}$                | 7.75 (1)                                                    | 1.26 (4)            | 5.35 (0)          | 1.05 (4)            |
| $\text{H}_2\text{CO} + h\nu \rightarrow \text{H}_2 + \text{CO}$               | 7.97 (1)                                                    | 1.30 (4)            | 4.84 (0)          | 9.86 (3)            |
| $\text{H}_2\text{CO} + \text{H} \rightarrow \text{H}_2 + \text{HCO}$          | 7.03 (0)                                                    | 1.45 (2)            | 6.16 (0)          | 1.22 (3)            |
| $\text{H}_2\text{CO} + \text{OH} \rightarrow \text{HCO} + \text{H}_2\text{O}$ | 8.04 (-2)                                                   | 4.81 (1)            | 9.26 (-2)         | 5.74 (2)            |
| $\text{H}_2\text{CO} + \text{O} \rightarrow \text{OH} + \text{HCO}$           | 1.94 (0)                                                    | 1.58 (3)            | 2.22 (0)          | 1.88 (4)            |
| Total                                                                         | 1.66 (2)                                                    | 2.74 (4)            | 1.87 (1)          | 4.10 (4)            |
| 3. Loss due to rainout                                                        |                                                             |                     |                   |                     |
|                                                                               | 1.62                                                        | 2.65 (2)            | 3.38 (-2)         | 7.17 (1)            |
| 4. Rain-out rate                                                              |                                                             |                     |                   |                     |
| $\text{H}_2 = 17 \text{ p.p.m.v.}$                                            | 5.5 (5)                                                     | 7.34 (7)            | 5.98 (3)          | 5.45 (7)            |
| $\text{H}_2 = 10^{-3} \text{ p.p.m.v.}$                                       | 8.6 (8)                                                     | 2.02 (9)            | 3.7 (8)           | 1.07 (10)           |

than unity if the flux is unity UV and that an increase of  $\text{CO}_2$  makes the situation only worse, as expected. If, however, the UV flux is increased as seen from Fig. 2, the situation changes radically, yielding a ratio of the order of unity or even larger at high altitudes, where the gas reactions simulated in ref. 61 are thought to occur. The increase of the UV flux has therefore distinctively improved the situation, as far as the  $\text{CO}/\text{CO}_2$  ratio is concerned.

The value for  $\text{H}_2\text{CO}$  computed in ref. 55 and corresponding to  $\text{UV} = 1$  and  $\text{CO}_2 = 1$  is here well reproduced within the uncertainties due to different photochemical models and numerical schemes. Our results indicate that the concentration of  $\text{H}_2\text{CO}$  is extremely sensitive (up to four orders of magnitude) to the abundance of  $\text{H}_2$ . Column densities are presented in Table 4. Perhaps the most interesting way of expressing the results concerning  $\text{H}_2\text{CO}$  is by way of Table 5, which gives the surface production and destruction rates. We also list the loss rates, two kinds of which are of interest. The first, representing the combined action of chemistry/photochemistry, represents a real destruction of  $\text{H}_2\text{CO}$  molecules. It is seen from Table 5 that the  $\text{H}_2\text{CO}$  destroyed at the surface exceeds its production there, that is  $\text{H}_2\text{CO}$  produced above the surface and transported downwards is also destroyed. The second type of loss due to

rainout, transfers  $\text{H}_2\text{CO}$  from the atmosphere to the ocean, thus preserving it for polymerization processes. Finally, in Table 5 we present the rainout rates, ( $\text{molecules cm}^{-2} \text{ s}^{-1}$ ) obtained by multiplying the values of Table 4 by the rainout coefficient. They depend critically on the values of  $\text{CO}_2$  and UV fluxes. The surface concentration presented in ref. 55,  $8.6 \times 10^8$ , (for  $\text{UV} = 1$  and  $\text{CO}_2 = 1$ ) can vary by a factor of  $10^4$  depending on the assumed values of  $\text{CO}_2$  and UV fluxes. Because the value given in ref. 55 was estimated to be sufficient to initiate polymerization processes in the ocean, we can conclude that if  $\text{H}_2\text{CO}$  were photochemically formed from  $\text{CO}_2$  and  $\text{H}_2\text{O}$ , in a UV-enhanced environment, the level of  $\text{CO}_2$  may have indeed been greater in the early atmosphere than it is today.

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# Phosphorylation of isocitrate dehydrogenase as a demonstration of enhanced sensitivity in covalent regulation

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*The sensitivity to regulation of proteins undergoing covalent modification can be greatly increased when the substrates saturate the converter enzymes. This phenomenon, termed zero-order ultrasensitivity, has been found to occur in the reversible phosphorylation of isocitrate dehydrogenase. The possibility that this enhanced sensitivity is a common feature of covalent regulatory systems is discussed.*

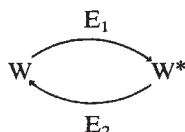
REGULATION by covalent modification is known to be involved in the control of almost every type of cellular process<sup>1,2</sup>. The phosphorylation cycle appears to be the most prevalent, but other types of covalent modification have important roles<sup>3</sup>. Protein phosphorylation has assumed increasing importance

with the discovery that the gene products of certain oncogenic viruses<sup>4,5</sup> and various receptor molecules, such as the epidermal growth factor receptor<sup>6</sup> and the insulin receptor<sup>7,8</sup>, have kinase activity.

An important feature of any control system is the degree to



which it is sensitive to changes in its environment. Such systems are typically regulated by internal controls, such as metabolite levels, and by external signals such as hormone concentrations, light and chemicals. The abruptness with which such systems can be turned on or off may be vital to the successful operation of the cell. Previous reports from this laboratory<sup>9,10</sup> have shown that covalent modification systems are theoretically capable of enhanced sensitivity to control if the converter enzymes are saturated with their substrates. This phenomenon was termed zero-order ultrasensitivity, to indicate that it arose from converter enzymes being in the saturation (zero-order) range. To illustrate this, we have plotted the velocity curves for two converter enzymes,  $E_1$  and  $E_2$ , which catalyse the interconversion of a substrate protein between modified ( $W^*$ ) and unmodified ( $W$ ) forms:



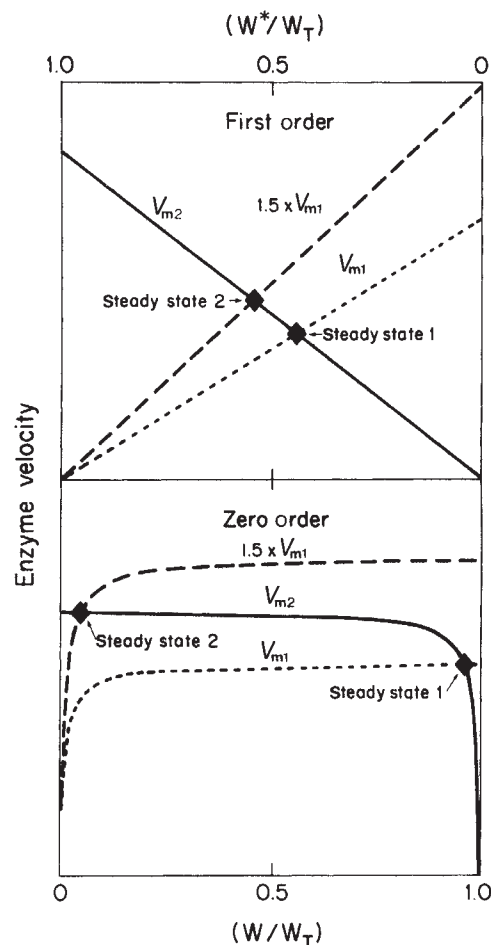
When these enzymes are in their first-order region (Fig. 1a), a 1.5-fold increase in the maximum velocity of  $E_1$  yields a modest change in the level of  $W$  modification at steady state. If, however, the enzymes are in the zero-order region (Fig. 1b), the same increase in the maximum velocity of  $E_1$  is seen to produce a profound change in the position of the steady state. The change from  $W$  to  $W^*$  thus becomes extremely sensitive to small changes in effectors of  $E_1$  or  $E_2$ .

Although the conditions for zero-order ultrasensitivity were defined mathematically, no experimental example had been observed. During a study of the glyoxylate bypass in *Escherichia coli*, we decided to examine the reversible phosphorylation of isocitrate dehydrogenase (IDH) to determine if it exhibited zero-order ultrasensitivity. The glyoxylate bypass, discovered by Kornberg<sup>11</sup>, occurs at a key branchpoint which allows *E. coli* to achieve net synthesis of cellular constituents during growth on acetate. Without this alternative pathway, nearly all the carbon would be lost as  $CO_2$  in the Krebs cycle. The branchpoint was shown to be regulated, at least in part, by a reversible change in IDH activity<sup>12</sup>, which was shown to correlate with phosphorylation of IDH<sup>13</sup>. The kinase and phosphatase responsible for the phosphorylation cycle have been purified to homogeneity and appear to be physically associated, possibly existing on a single polypeptide chain<sup>14</sup>.

We report here an experimental demonstration of zero-order ultrasensitivity. This was accomplished by analysing the regulation of steady-state phosphorylation when the substrate protein, IDH, was present at concentrations either well above or well below the Michaelis-Menten constant of IDH phosphatase. In addition, a complete analysis required that we determine the stoichiometry of phosphorylation and the kinetic behaviour of the individual converter enzymes.

## Stoichiometry of phosphorylation

The stoichiometry of phosphorylation and the effect on IDH activity were determined by following the time course of the kinase reaction (Fig. 2). Reaction conditions were selected to yield quantitative phosphorylation. Garnak and Reeves<sup>13</sup> demonstrated a correlation between a decrease in IDH activity and phosphorylation *in vivo*, but the extent of the inactivation was not known. However, simultaneous monitoring of the incorporation of  $^{32}P$ -phosphate and inhibition of IDH activity in the *in vitro* system could provide a precise estimate of the activity of the phosphorylated protein. The superimposability of the two curves shown in Fig. 2 indicates that the phosphoprotein was essentially inactive. More detailed experiments have shown that the phosphorylated protein actually retains less than 0.5% of the activity of the dephosphoprotein. The stoichiometry of the phosphorylation reaction was 1 mole of phosphate per mole of IDH monomer. Similar results have been obtained by H. G. Nimmo (personal communication). The activity of the protein

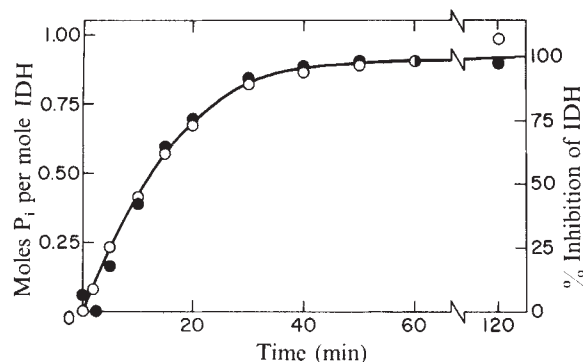


**Fig. 1** Zero-order ultrasensitivity in a theoretical modification cycle. The velocity curves for two Michaelis-Menten enzymes,  $E_1$  and  $E_2$ , which catalyse the interconversion of proteins  $W$  and  $W^*$  (see text), are plotted as a function of their normalized substrate concentration. The normalized Michaelis-Menten constants, defined as the Michaelis-Menten constant divided by the total concentration of  $W(W_T)$ , were set to either 100 (a), yielding a first-order system, or to 0.01 (b), yielding a zero-order system. The maximum velocity of  $E_2(V_{m2})$  was taken to be 1 (arbitrary units) while that for  $E_1(V_{m1})$  was either 0.8 or 1.2, as indicated. As the system achieves steady state when the velocity of the converter enzymes are equal, the fractional modification of  $W$  at steady state is given by the intersection of the velocity curves of the opposing enzymes. When the converter enzymes are both in their first-order regions (a), a 1.5-fold increase in the maximal velocity of  $E_1$  has a fairly mild effect on the fractional modification (steady state 2 versus steady state 1). If, however, the converter enzymes are in their zero-order regions, this same 1.5-fold increase in the maximum velocity of  $E_1$  produces a dramatic change in fractional modification (b).

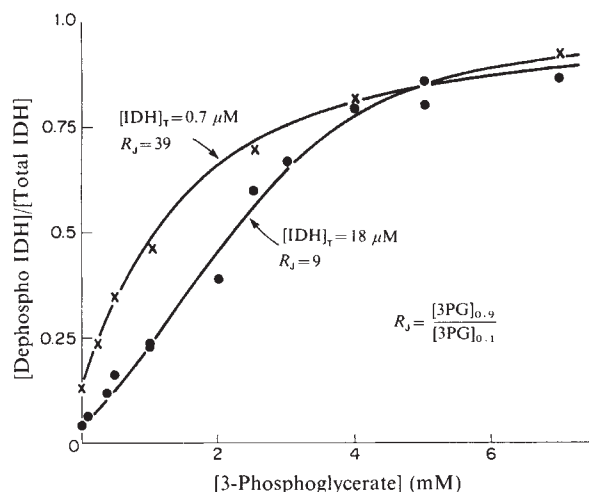
is completely restored by dephosphorylation catalysed by the phosphatase.

## Steady-state phosphorylation level

In the living cell, the extent of phosphorylation is determined by the kinetic behaviour of the opposing kinase and phosphatase reactions. Figure 3 shows the effect of 3-phosphoglycerate, which activates the phosphatase and inhibits the kinase, on the steady-state phosphorylation of IDH. The reaction mixture initially contained dephospho-IDH, kinase/phosphatase, ATP and 3-phosphoglycerate in addition to other buffer constituents. The reaction was begun by addition of kinase/phosphatase. The phosphorylation state of IDH was then monitored by periodically assaying for IDH activity. As the phospho form of IDH is inactive, IDH activity is an indication of the fraction of IDH in the dephospho form. Two very different concentrations of the phosphorylated protein, IDH, were chosen. At the lower



**Fig. 2** Time course of the phosphorylation reaction. The progress of the kinase reaction was followed by simultaneously monitoring incorporation of phosphate (○) and inhibition of IDH activity (●). Each sample contained 25 mM *N*-morpholinopropane sulphonate, pH 7.5, 200  $\mu$ M [ $\gamma$ - $^{32}$ P]ATP (114 c.p.m.  $\mu$ mol $^{-1}$ ), 5 mM MgCl $_2$ , 0.4 mg ml $^{-1}$  bovine serum albumin, 1 mM dithiothreitol, 100 mM NaCl, 70 pmol of dephospho-IDH and 0.04 U ml $^{-1}$  of purified IDH kinase<sup>14</sup> in 50  $\mu$ l final volume. Dephospho-IDH was purified by a modification of the method of Garnak and Reeves<sup>20</sup>. The reaction was begun by adding [ $\gamma$ - $^{32}$ P]ATP and then incubating at 37 °C. At the indicated times, the reaction was stopped by addition of 10  $\mu$ l of 50 mM ATP, 100 mM EDTA, pH 7.5, and placed on ice. IDH activity was determined spectrophotometrically in 25 mM *N*-morpholinopropane sulphonate, pH 7.5, 5 mM MgCl $_2$ , 250  $\mu$ M NADP, 500  $\mu$ M D,L-isocitrate. Incorporation of phosphate was determined by spotting aliquots of the reaction mixture onto filter paper squares, followed by isolation of the labelled product, as described by Reimann *et al.*<sup>21</sup>. Phosphorylation is expressed as moles of phosphate per mole of IDH monomer.



**Fig. 3** Effect of 3-phosphoglycerate on the steady-state phosphorylation of IDH. Regulation of the steady-state phosphorylation cycle was monitored at 0.7 (x) and 18  $\mu$ M (●) total IDH. The reaction mixtures contained buffer A (25 mM *N*-morpholinopropane sulphonate, pH 7.5, 6 mM MgCl $_2$ , 100 mM NaCl, 1 mM dithiothreitol, 2 mg ml $^{-1}$  bovine serum albumin, 1 mM D-isocitrate, 1 mM EDTA, 2 mM phosphocreatine, 10 U ml $^{-1}$  creatine phosphokinase) in addition to 2 mM ATP, and the indicated amounts of dephospho-IDH, expressed as concentration of the monomer, and 3-phosphoglycerate in a final volume of 50  $\mu$ l. The reaction was begun by addition of purified kinase/phosphatase to 2.2 U ml $^{-1}$  (ref. 14) and then incubated at 37 °C. At  $\frac{1}{2}$ -h intervals, aliquots were withdrawn and assayed for IDH activity, as described in Fig. 2 legend. The steady state was achieved by the first time point, and this was confirmed by the second. Since the phospho form of IDH is inactive, the decrease in IDH activity was a direct measure of the fractional modification of IDH at steady state. The curves were determined by a computer-generated best fit using the rate laws determined in initial velocity experiments as described in the text. The sensitivity coefficients were determined from the slope of the curve at 50% of the maximum effect.

concentration, 0.7  $\mu$ M, both converter enzymes were essentially in their first-order regions. At 18  $\mu$ M the phosphatase was nearly saturated with phospho-IDH over most of the titration curve.

At a total IDH concentration of 0.7  $\mu$ M, 13% of the IDH was in the dephospho form in the absence of 3-phosphoglycerate, and approached 90% at the upper limit of the titration. Fifty per cent of this effect was achieved at 1.2 mM 3-phosphoglycerate. When the total IDH concentration was increased to 18  $\mu$ M, the percentage IDH in the dephospho form was initially 4%, and ~90% at the limit, with half the effect observed at 2.3 mM 3-phosphoglycerate.

An apparent increase in the sigmoidicity of the curve at the higher IDH concentration was an indication that a zero-order effect was being observed. To quantitate this effect, the sensitivity coefficient  $R_s$  was calculated for both curves.  $R_s$ , which has been used to evaluate the sensitivity of allosteric proteins<sup>15</sup>, is defined as the ratio of the concentration of effector needed to produce a 90% response to that producing a 10% response (see Fig. 3). This ratio will be 81 for a simple hyperbolic system, less than 81 for a system with enhanced sensitivity and greater than 81 for a system which is less sensitive. At 0.7  $\mu$ M total IDH, the sensitivity coefficient is 39, indicating a moderate increase in sensitivity relative to a hyperbolic system. When the total IDH concentration was increased to 18  $\mu$ M, the coefficient is 9, indicating a substantial increase in sensitivity to effector control. A value of 9 for  $R_s$  for an allosteric protein would correspond to a Hill coefficient of 2.0.

The interpretation of these results seemed to be straightforward. The increased sensitivity relative to Michaelis-Menten ( $R_s = 39$  compared with  $R_s = 81$ ) at 0.7  $\mu$ M total IDH is due to the multi-step effect; that is, the simultaneous activation of the phosphatase and inhibition of the kinase by 3-phosphoglycerate (see below). The increased sensitivity ( $R_s = 9$ ) at the higher IDH concentration seems to be due to a combination of multi-step effect and the increased sensitivity due to saturation of the kinase or phosphatase. Rigorous analysis of this case required, however, that the behaviour of the individual converter enzymes be defined.

## Kinetics of kinase and phosphatase

The effect of 3-phosphoglycerate (3PG) on the kinetic behaviour of the phosphatase is shown in Fig. 4. As can be seen, the phosphatase is activated by 3-phosphoglycerate via an increase in the maximum velocity without an appreciable effect on the Michaelis-Menten constant. The rate law is given by

$$v_p = \frac{[IDH-P] V_p (K_A + \beta[3PG])}{K_p + [IDH-P]} \quad (1)$$

where  $V_p$  and  $K_p$  are the maximum velocity and the Michaelis-Menten constants, describing basal activity, while  $K_A$  and  $\beta$  are, respectively, the activation constant and maximum fold activation observed for 3-phosphoglycerate. The values of these parameters in the presence of 1 mM D-isocitrate are given in Table 1. Isocitrate was added at this concentration because it activates the phosphatase and inhibits the kinase and so allows 3-phosphoglycerate to achieve greater completion of the steady-state titration curve. In the conditions used in the steady-state experiments, the kinase is essentially in its first-order range with respect to IDH. Its kinetic behaviour can be characterized by a pseudo-first-order rate constant for dephospho-IDH and an inhibition constant for 3-phosphoglycerate (Table 1).

## Computer fit

If the increased sensitivity at the higher IDH concentration is due to zero-order ultrasensitivity, the intrinsic enzyme constants ( $K_m$ ,  $V_m$ , etc.) should not vary at the different IDH levels and should correspond to those determined by initial velocity measurements. Once the rate laws for the kinase and phosphatase had been determined, it was possible to pursue a computer-generated best fit for the steady-state experiments. A



least-squares criterion and a requirement of a random distribution of residuals were used to fit the theoretical curves of the steady states to the experimental data (Fig. 3). Values close to the kinetic constants determined by the initial velocity experiments were given as a starting point for the least-squares procedure, and the computer program then selected the optimal values which fitted the steady-state data. These parameter values were used to generate the curves shown in Fig. 3. Although the number of parameters involved does not allow a unique solution, the criteria used impose a major constraint on the solution. As such, the close agreement obtained by the curve-fitting procedure between the steady-state data and the parameters determined directly from initial velocity experiments (Table 1) indicates that we can accurately describe the behaviour of the converter enzymes in the steady-state experiment. Most importantly, it shows that the effective kinetic constants do not shift on going from low to high values of IDH concentration. Thus, the difference in sensitivity of the two curves results from a zero-order effect rather than from alteration of the values of the enzymatic constants.

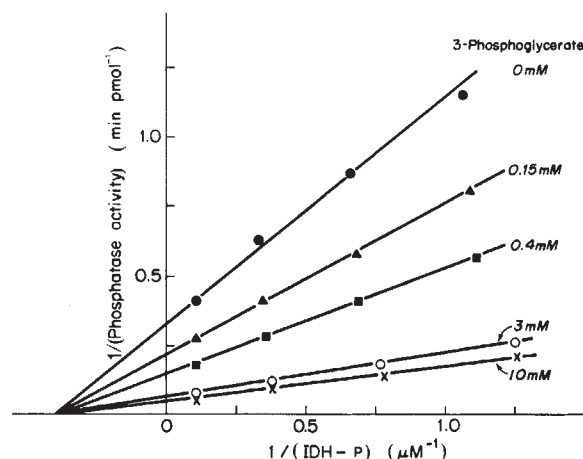
It might be pointed out that, when the total IDH is increased to  $18 \mu\text{M}$ , the fraction of IDH in the dephospho form is decreased over most of the titration curve. This is precisely the result expected if the phosphatase was approaching saturation with substrate at the higher IDH concentration, while the kinase was not. Thus, this observation lends additional support to the compatibility of the initial velocity and steady-state measurements.

## Reasons for increased sensitivity

A graphic illustration of the increased sensitivity to regulation is given in Fig. 5, in which the regulation observed at  $18 \mu\text{M}$  total IDH is compared with a simple hyperbola. To simplify the comparison of the two systems, the relative increase in 3-phosphoglycerate needed to increase the response from 0.1 to 0.9 of the maximum is shown at the top of the figure. Although an 81-fold increase in 3-phosphoglycerate concentration would

have been necessary for a hyperbolic system, at  $18 \mu\text{M}$  total IDH only a ninefold increase was needed.

The observed increase in the sensitivity of the IDH phosphorylation cycle to regulation could arise either from a multi-step effect or a zero-order effect. The multi-step effect is defined as the same effector acting at several steps in a cascade.

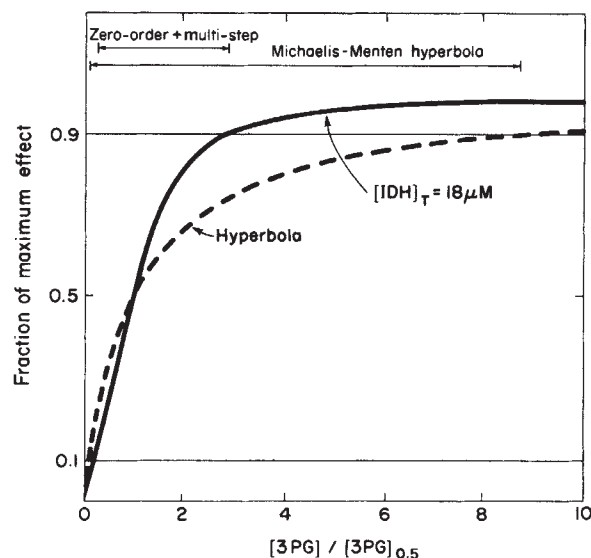


**Fig. 4** Activation of the phosphatase by 3-phosphoglycerate. The reaction mixture contained buffer A (see Fig. 3 legend), 2 mM ATP and the indicated amounts of phospho-IDH, expressed as concentration of the monomer, and 3-phosphoglycerate in a final volume of  $50 \mu\text{l}$  (the phospho-IDH had been prepared *in vitro* followed by purification of the product by Affigel blue chromatography). The reaction was begun by addition of purified kinase/phosphatase to  $0.5 \text{ U ml}^{-1}$  (ref. 14), incubated for 5 min at  $37^\circ\text{C}$ , terminated by addition of  $100 \mu\text{l}$  of 20% trichloroacetic acid and placed on ice. Precipitated protein was removed by centrifugation and  $100\text{-}\mu\text{l}$  aliquots of the supernatant were removed for determination of  $^{32}\text{P}$  release.

**Table 1** Kinetics of isocitrate dehydrogenase phosphorylation

|                                             | Independent measurements | Computer fit to steady state<br>$0.7 \mu\text{M}$ IDH | $18 \mu\text{M}$ IDH |
|---------------------------------------------|--------------------------|-------------------------------------------------------|----------------------|
| <b>Kinase</b>                               |                          |                                                       |                      |
| Rate constant ( $\text{min}^{-1}$ )         | 0.30                     | 0.32                                                  | 0.33                 |
| $K_i$ for 3PG (mM)                          | 1.2                      | 1.5                                                   | 1.0                  |
| <b>Phosphatase</b>                          |                          |                                                       |                      |
| $V_{\text{max}}$ ( $\mu\text{M min}^{-1}$ ) | 0.25                     | 0.19                                                  | 0.25                 |
| $K_m$ ( $\mu\text{M}$ )                     | 2.6                      | 3.0                                                   | 3.0                  |
| Max. activation                             | 10.2                     | 10.1                                                  | 11.0                 |
| $K_{\text{act}}$ for 3PG (mM)               | 3.1                      | 3.2                                                   | 3.4                  |
| <b>Steady state</b>                         |                          |                                                       |                      |
| $R_j$                                       |                          | 39                                                    | 9.1                  |

Independent measurements were determined from initial velocity measurements as described in the text. The computer fit was determined from steady-state curves shown in Fig. 3. The rate laws observed in initial velocity experiments were used as starting points and final values for kinetic constants ascertained by computer using the least-squares criterion. Kinase assays consisted of buffer A (Fig. 3) containing  $1 \text{ mM}$  [ $\gamma\text{-}^{32}\text{P}$ ]ATP ( $150 \text{ c.p.m. pmol}^{-1}$ ),  $6 \mu\text{M}$  dephospho-IDH and varying concentrations of 3-phosphoglycerate (3PG) in  $50 \mu\text{l}$  final volume. The reaction was begun by adding kinase/phosphatase to  $0.05 \text{ U ml}^{-1}$ . The mixture was incubated for 25 min at  $37^\circ\text{C}$ . The reaction was stopped and the product isolated as described in Fig. 2 legend. The inhibition constant was determined by Dixon plot<sup>22</sup>. The pseudo-first-order rate constant was determined from the sample without 3-phosphoglycerate and is reported for the concentration of kinase/phosphatase used in the steady-state experiment (Fig. 3). The kinase activity has been found to be linear with protein. Phosphatase values were calculated from the results presented in Fig. 4. The value of the  $V_{\text{max}}$  is calculated for the concentration of kinase/phosphatase used in the steady-state experiment (Fig. 3). The phosphatase activity has been found to be linear with protein in this range.  $R_j$  was calculated from the relative slope of the computer fit curve at 50% of the effects, as described in the text.



**Fig. 5** Sensitivity of the steady-state phosphorylation cycle to regulation. The steady-state curve observed at  $18 \mu\text{M}$  total IDH is compared with a simple hyperbola. To aid the comparison, the steady-state curve is plotted as a fraction of the maximum effect versus normalized 3-phosphoglycerate concentration. This curve was computer-generated using the kinetic constants given in Table 1 which had generated the steady-state curve in Fig. 3. For comparison, a simple hyperbola, intersecting the steady-state curve at the half-maximal effect, is also plotted. The relationship between the sensitivity coefficients, defined as the relative increase in 3-phosphoglycerate needed to increase the fraction of the maximum effect from 0.1–0.9, is shown graphically at the top of the figure. The label 'zero-order + multi-step' refers to the steady-state curve determined at  $18 \mu\text{M}$  total IDH.

In this case it refers to the simultaneous activation of the phosphatase and inhibition of the kinase. The multi-step effect will contribute to the regulatory sensitivity at any IDH concentration, whereas the zero-order effect has been shown to occur only when one or both of the converter enzymes are saturated with substrate<sup>9</sup>.

The quantitative contribution of the multi-step effect can thus be evaluated at low IDH concentrations. When both converter enzymes are in their first-order range with respect to their protein substrates, the steady state can be described by

$$\frac{[\text{IDH}]}{[\text{IDH}] + [\text{IDH-P}]} = \frac{f_{3\text{PG}}}{f_{3\text{PG}} + (k_K K_P / V_P)} \quad (2)$$

where  $k_K$  is the pseudo-first-order rate constant of the kinase and  $V_P$  and  $K_P$  are the maximal velocity and Michaelis-Menten constants of the phosphatase. The term  $f_{3\text{PG}}$  describes the regulation of the kinase and phosphatase and is given by

$$f_{3\text{PG}} = \left( \frac{K_A + \beta[3\text{PG}]}{K_A + [3\text{PG}]} \right) \left( \frac{K_i + [3\text{PG}]}{K_i} \right) \quad (3)$$

where  $K_i$  is the inhibition constant for the kinase and the other terms are as defined above.

It might seem intuitively that the simultaneous activation of one step and inhibition of an opposing step will yield a sensitivity proportional to the square of the effector concentration. As can be seen from equation (3), this maximum value would only be realized if the effector concentration were much greater than  $K_i$  and much less than  $K_A$  and if the value of  $\beta$  were very large. Although such circumstances might be selected for by evolutionary pressure, the conditions required are very extreme. As such, they may not be found very often and, in fact, are not found in this case. Nevertheless, the multi-step effect does make a small contribution to the sensitivity, yielding a value of 39 for  $R_j$  at 0.7  $\mu\text{M}$  IDH.

The only difference in conditions between the steady-state experiments performed at 0.7 and 18  $\mu\text{M}$  total IDH is the total concentration of IDH, yet the sensitivity of the system to control is much greater at the higher concentration. The difference is caused by the introduction of zero-order ultrasensitivity at the higher concentration of IDH. As described above, the intrinsic enzyme constants do not change, so a small contribution to sensitivity is made by the multi-step effect. The main effect, however, on increasing the concentration of IDH to 18  $\mu\text{M}$  results from the phosphatase being saturated over much of the titration curve and so introducing zero-order ultrasensitivity.

## Relationship to the living cell

To maintain compatibility between the *in vitro* experiments and the living cell, reaction components and concentrations were chosen to be at near physiological conditions. The cellular level of 3-phosphoglycerate is 2.5 mM (K. Walsh and D.E.K., unpublished results), and so the concentrations of this effector used *in vitro* were within the appropriate range. The ratio of kinase to phosphatase was readily maintained at the physiological value as they appear to be associated as a single protein<sup>14</sup>. The D-isocitrate concentration, however, was maintained at 1 mM, well above the physiological level of 160  $\mu\text{M}$  (ref. 16 and K. Walsh

and D.E.K., unpublished results). When the steady-state experiments were performed at 100  $\mu\text{M}$  D-isocitrate, a clear zero-order effect was observed, but the quantitation was less accurate because the titration curve could not be brought to completion within a reasonable range of 3-phosphoglycerate. The cellular concentration of IDH is  $\sim 35 \mu\text{M}$ , double the concentration yielding zero-order ultrasensitivity in the *in vitro* experiments. Thus, an even greater zero-order effect than that shown here would be anticipated *in vivo*.

## Discussion

This report represents the first experimental observation of zero-order ultrasensitivity, which had been predicted from theoretical considerations<sup>9</sup>. Although the extent to which this phenomenon is found with other systems is unknown, this is the first system which has been examined for this effect, and the system was not selected because of any special characteristics. It seems more likely that it was found in this case because zero-order ultrasensitivity is prevalent in nature than that this is an extraordinary coincidence. It has been found, for example, that the phosphatase which dephosphorylates glycogen phosphorylase in rabbit skeletal muscle has a  $K_m$  for the phosphoprotein of 2–5  $\mu\text{M}$ , while the tissue concentration of its substrate is  $\sim 100 \mu\text{M}$  (refs 17, 18). Thus, other systems satisfy the *prima facie* requirements of zero-order ultrasensitivity, a concentration of substrate protein substantially greater than the Michaelis-Menten constant of the converter enzyme. Such a substrate protein need not be at high concentrations *per se*. It is only necessary that its concentration substantially exceeds the Michaelis-Menten constants of the converter enzymes. Although the example reported here involves phosphorylation of an enzyme, zero-order effects are not limited to interconvertible enzymes or to phosphorylation. There is equivalent potential for other substrate proteins, such as ion channels and sensory receptors, and for other forms of covalent modification, such as reversible methylation.

We emphasize that the zero-order effect is a property of steady-state systems and would not be possible if there were a simple equilibrium between the two forms of the protein. It results from the kinetic characteristics of opposing, energy-driven reactions. This flow of energy is, in fact, the price the cell must pay for an increased sensitivity to environmental changes.

The physiological consequences of such a phenomenon may have great significance. Some systems may show threshold effects giving abrupt on/off switching and others may show milder increases just as some proteins are strongly cooperative and others mildly cooperative. This could have significant consequences in both normal and pathological states. For example, it has been suggested that several viral transforming genes code for protein kinase activities having the same substrate as a normal cellular kinase<sup>19</sup>. If the cellular level of this interconvertible substrate were in the zero-order region of the enzymes of the phosphorylation cycle, even modest increases in kinase activity could result in substantial changes in the phosphorylation state of the substrate. If the substrate protein had a key role in cellular regulation, such a major change in its phosphorylation state could have pathological consequences.

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## Simultaneous acceleration of electrons and ions in solar flares

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Two very intense and impulsive nuclear  $\gamma$ -ray and electron bremsstrahlung X-ray emitting solar flares, on 7 and 21 June 1980, were observed with  $<2$  s time resolution. These observations have allowed us to study, for the first time, the relative starting times of energetic ion and electron interactions with the solar atmosphere. The simultaneous starting times of X rays  $>40$  keV and  $\gamma$ -ray emission show that electrons and ions were accelerated within seconds of each other. The near simultaneous peaking, together with the observation of  $>400$ -MeV neutrons, show that the solar flare acceleration process can produce the full intensity and spectrum of electrons ( $>1$  MeV) and ions ( $>10^2$  MeV) in a time scale of  $<5$  s. These results are in direct contradiction to the widely accepted concept of solar flare particle acceleration.

It has been assumed that the most likely acceleration process for electrons and ions in solar flares involves two phases<sup>1</sup>. In the first phase, only electrons are accelerated which produce the well-observed impulsive hard X-ray emissions at energies  $<100$  keV. This phase also provides the particle injection process for the second phase during which ions and relativistic electrons are accelerated over time scales of minutes. One of the features that makes this concept very attractive is that it allows for a slower process, such as stochastic Fermi acceleration, for the very energetic ions and electrons<sup>2</sup>.

The flares discussed here are the first 2 of more than 20  $\gamma$ -ray line emitting flares observed by the  $\gamma$ -ray spectrometer (GRS)<sup>3</sup> on the Solar Maximum Mission Satellite (SMM) which was launched on 14 February 1980. Results from these two flares include observations of the 2.223-MeV line from  $^1\text{H}(n, \gamma)^2\text{H}$  (refs 4, 5), the 0.511-MeV line from  $e^+e^-$  annihilation<sup>6</sup>, prompt nuclear lines at 4.44 and 6.13 MeV from  $^{12}\text{C}^*$  and  $^{16}\text{O}^*$  (refs 6, 7), and the first observation of energetic solar neutrons at 1 AU (ref. 8).

Figure 1 shows the time history of the count rates in four energy bands from 40 keV to 6.4 MeV for the 7 June 1980 event. Forrest *et al.*<sup>9</sup> have studied the spectral properties of this event and have shown that the count rate in the energy band 4.1–6.4 MeV is dominated by ion-induced broad and narrow  $\gamma$ -ray lines from CNO, while below  $\sim 350$  keV the count rate is dominated by electron bremsstrahlung. Figure 2 shows a similar set of time histories extending from 40 keV to  $>25$  MeV for the 21 June 1980 event. Again, the presence and intensity of the 4.43- and 6.13-MeV lines<sup>6</sup> show that the count rate in the 4.1–6.4-MeV band is largely dominated by  $\gamma$ -ray lines from CNO. In this same event, Chupp *et al.*<sup>8</sup> have shown that the only reasonable production interval for the detected neutrons  $>50$ –600 MeV is during the  $\gamma$ -ray peaks observed in the impulsive phase. The time profile of the 2.223-MeV line<sup>5</sup>, which is produced by the low-energy portion of the neutron spectrum, is consistent with this interpretation. We conclude that Fig. 1a–c represents the X-ray emission time profile produced by an electron spectrum extending up to  $>400$  keV, and Fig. 1d represents the prompt  $\gamma$ -ray line emission time profile produced by energetic ions. In a similar way Fig. 2a, b shows the interaction time profile of energetic electrons while Fig. 2c shows that of energetic ions  $>10$  MeV. Figure 2d shows the interaction time profile for electrons  $>25$  MeV and/or that of energetic ions with energies above the  $\pi^0$  production threshold of  $>300$  MeV.

There are two important points. First, both a visual inspection and the result of a statistical test of the data show that the start of the observed excess emission is coincident in time ( $\pm 2.2$  s

for 7 June and  $\pm 0.8$  s for 21 June), for all energy bands in both flares. This result sets severe constraints on any time delay between electron- and ion-acceleration processes, as well as on the physical separation between the acceleration and the interaction sites. Second, there is clear evidence that the times of the peak count rates for the ion-induced emissions are sometimes delayed by a few seconds from the corresponding maximum of the electron-induced emissions. In the past, the absence of perfect simultaneity in the peak and decay times of the  $\gamma$ -ray and X-ray emissions has been interpreted in terms of second phase<sup>10</sup> and second step<sup>11</sup> acceleration. However, X-ray and  $\gamma$ -ray emission time profiles result from a convolution of both the acceleration and the energy loss processes and information on the time dependence of the acceleration process can be obtained only by solving a continuity equation<sup>12,13</sup>. Small time differences can be explained by any one of several processes which must control in different ways the injection, the propagation and the slowing down of the two species of particles. As an example, S. Enome (personal communication) shows that the difference in peak times between the X-ray bands in these two events and the  $\gamma$ -ray bands can be explained by the energy loss process in a high-density, thick-target production region. On the other hand, Nakajima *et al.*<sup>14</sup> have compared the GRS SMM X-ray and  $\gamma$ -ray time profiles from these two flares with the corresponding 17-GHz

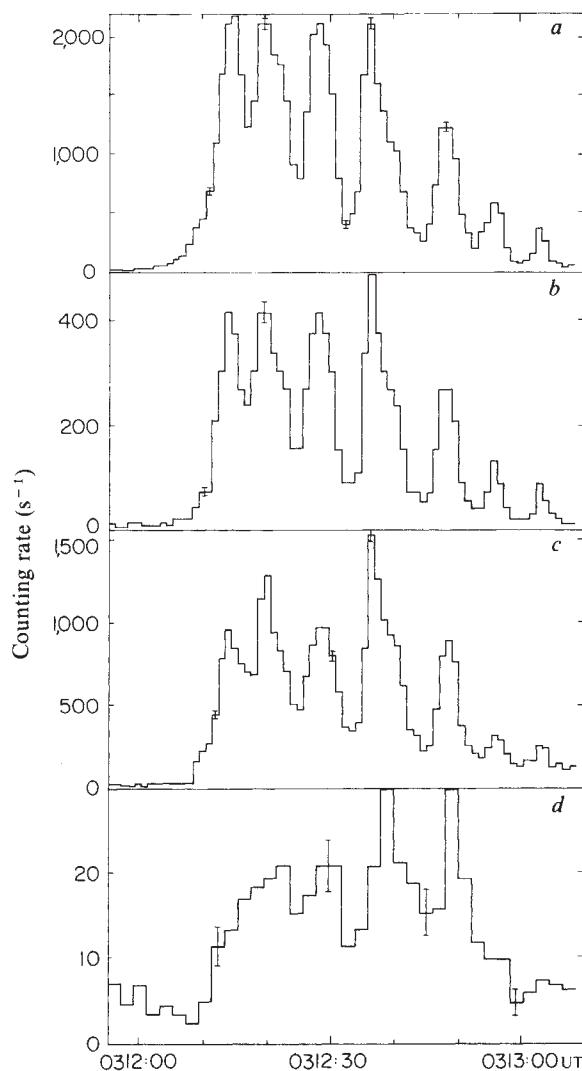
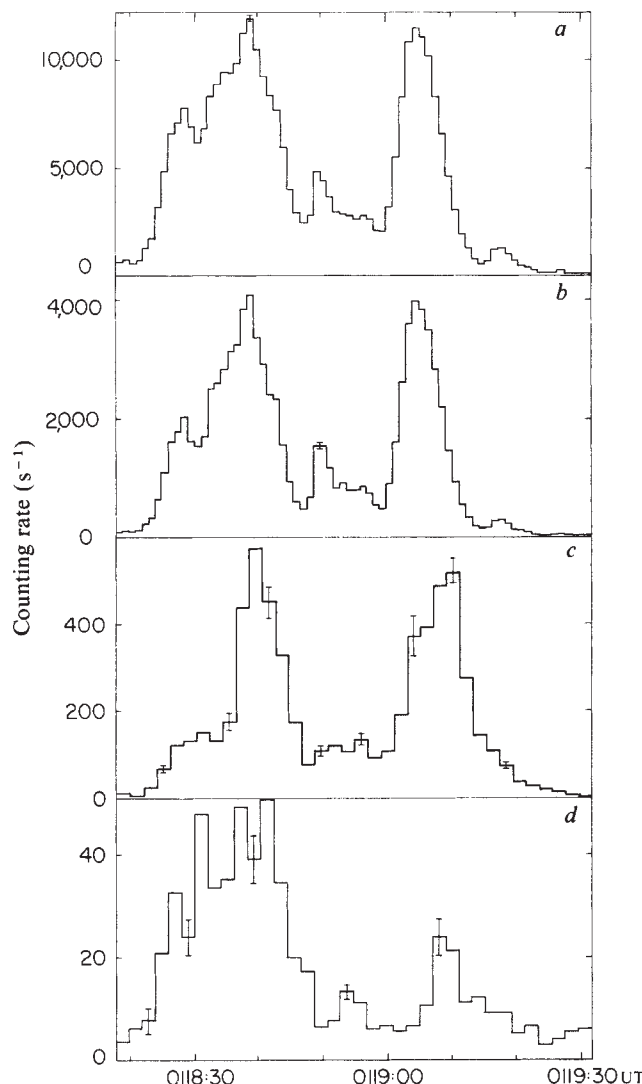


Fig. 1 Counting rate time histories in four energy bands: a, 40–80 keV; b, 80–140 keV; c, 330–380 keV; d, 4.1–6.4 MeV observed with the GRS on 7 June 1980. The typical ( $\sim 1\sigma$ ) errors shown are due to counting statistics.



**Fig. 2** Counting rate time histories in four energy bands: *a*, 40–80 keV; *b*, 80–140 keV; *c*, 4.1–6.4 MeV; *d*, >25 MeV observed with the GRS on 21 June 1980. These rates are corrected for instrument live time losses and the ( $\sim 1\sigma$ ) errors are due to counting statistics.

flux time profiles observed at the Nobeyama Observatory, Japan. They conclude that each intensity maximum seen in X rays and  $\gamma$  rays (see Figs 1 and 2) is actually composed of two shorter and not fully resolved injections with different electron-to-ion number ratios and/or injection properties and hence may suggest two phases of acceleration. We feel that this interpretation ignores the real possibility of a time-dependent emission and absorption effect which may control the microwave profile at the maximum peak emission such as observed by Kaufmann *et al.*<sup>15</sup>. Therefore, the simultaneous occurrence of peak rates in microwave, X rays and  $\gamma$  rays, when observed, may be coincidental.

We conclude that the simultaneous starting times of the X-ray and  $\gamma$ -ray observations presented here are in clear conflict with the view that ions can be accelerated only in a delayed ( $>1$  s) second phase (step) process. While these observations cannot distinguish between one common acceleration process for both electrons and ions as compared with two separate processes operating in near unison, we feel that these data can be simply explained by a single process. The small, but real time difference of the maximum count rates between X rays and  $\gamma$  rays then provides information on the injection and energy loss mechanisms, rather than the acceleration process. We believe that the information required to decide between one common or two different processes for acceleration of electrons and ions will

not come from studies of single flares, but rather from statistical study of a large number of hard X-ray and  $\gamma$ -ray flares. We predict that if a common process is responsible for accelerating both electrons and ions, then the smaller observed frequency of  $\gamma$ -ray flares will be due to differences in detector flux sensitivity limits for X rays and  $\gamma$  rays. However, if two different processes are operative, we would be more likely to expect a threshold effect such that  $\gamma$  rays will be produced only in larger flares. Hudson<sup>16</sup> has carried out such a study with solar cosmic ray (SCR) events and claims to have found such a threshold effect. However, at present it is not clear if the ion population measured as SCRs near 1 AU is the same as that which produces the  $\gamma$ -ray emissions discussed here. In fact, preliminary studies of SCRs from  $\gamma$ -ray flares show little correlation<sup>17,18</sup>. It may be that a secondary acceleration process does operate sometimes, but only to produce SCRs. In any case, the need to find a primary flare process(es) which can accelerate electrons to  $>1$  MeV and ions to  $>10$  MeV in time scales of  $<5$  s remains.

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## Successive electron and ion accelerations in impulsive solar flares on 7 and 21 June 1980

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**A study of the relative timing of solar flare emissions at different wavelengths can provide valuable insights into the associated particle-acceleration mechanisms. We report here that the significant systematic differences between the times of occurrence of the peak intensities of microwave, hard X-ray and  $\gamma$ -ray line emissions, which were found in two impulsive flares on 7 and 21 June 1980 at 0312 and 0118 UT respectively, suggest that two kinds of acceleration take place successively within a few seconds.**

The time profiles of the impulsive burst on 7 June are shown in Fig. 1. The burst is composed of seven successive pulses of a quasiperiodicity of  $\sim 8$  s, for each of which the hard X-ray,  $\gamma$ -ray and microwave emissions were observed almost simultaneously<sup>1–6</sup>.



Hard X-ray and  $\gamma$ -ray observations were made with the  $\gamma$ -ray spectrometer (GRS) aboard the Solar Maximum Mission Satellite (SMM) (Fig. 1a-c). From the comparison of the time profiles, Chupp<sup>3</sup> has reported that the prompt  $\gamma$ -ray line emission peak (4.1–6.4 MeV; mainly due to de-excitation of excited  $^{12}\text{C}$  and  $^{16}\text{O}$  produced by interaction of ions  $>30$  MeV) lags the hard X-ray emission peak (40–140 keV and 305–355 keV) by a few seconds.

Microwave observations were made using the 17-GHz polarimeter at Nobeyama. The time profile resembles those of hard X rays and  $\gamma$  rays. However, at least two of the microwave pulses (numbers 2 and 4 in Fig. 1) are composed of two sub-peaks. The first microwave sub-peak coincides with the hard X-ray peak while the second coincides with the prompt  $\gamma$ -ray line peak. This correspondence may also be present in the third pulse (number 3 in Fig. 1).

The position and size of the microwave burst source are derived from records obtained using the 17-GHz interferometer at Nobeyama<sup>7</sup>. The source position shows no significant shift  $>3$  arc s between the two sub-peaks. The source height is lower than 10 arc s above the corresponding H $\alpha$  flare. The source size is  $<7$  arc s FWHM in the east-west direction. This value indicates that the peak brightness temperature is at least of the order of  $10^9$  K if the source shape is circularly symmetric.

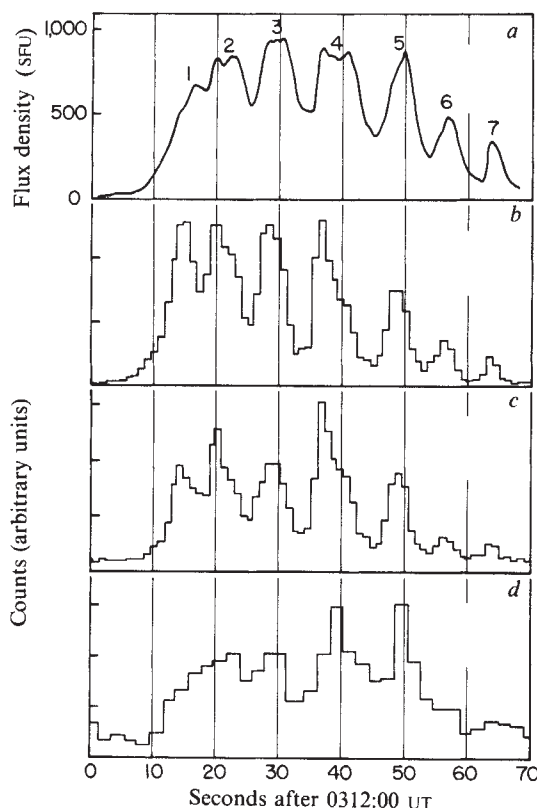
The 21 June 1980 burst is composed of four successive pulses as shown in Fig. 2. The relative timing between the peak intensities of the three emissions is essentially similar to that of the 7 June event. In fact, the  $\gamma$ -ray peak lags the hard X-ray peak by several seconds. This is clearly seen in the fourth pulse. Each microwave pulse is again composed of two sub-peaks. The hard X-ray peak corresponds to the first microwave sub-peak and the prompt  $\gamma$ -ray line peak to the second microwave sub-peak. Again no significant differences of position and size

are found between the source of the two microwave sub-peaks. The other characteristics of the microwave source are about the same as those of the 7 June event.

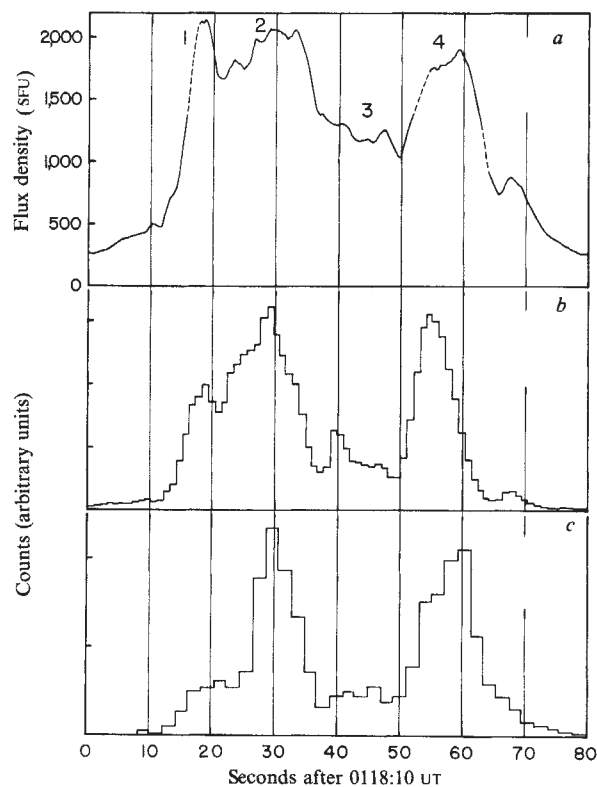
Both the two impulsive bursts discussed above consist of discrete pulses of several seconds duration. Many of the microwave pulses consist of two sub-peaks. The hard X-ray emission corresponding to the second microwave sub-peak is weaker than that corresponding to the first sub-peak. On the other hand, the  $\gamma$ -ray emission corresponding to the first microwave sub-peak is weaker than that corresponding to the second sub-peak. This is a systematic tendency seen in many discrete pulses.

A possible interpretation of the abovementioned tendency is that two kinds of acceleration take place repeatedly during the whole duration of the impulsive bursts. Electrons are preferentially accelerated at the time of the first microwave sub-peak which gives rise to the hard X-ray and microwave emissions, and a few seconds later both ions and electrons are accelerated together, giving rise to the  $\gamma$ -ray line and microwave emissions, respectively.

Why are the observed hard X-ray emissions much weaker at the time of the second microwave sub-peak? Within the framework of the particle acceleration model proposed above, we may consider the following possibilities. (1) Electrons are accelerated to relativistic energies at the time of the second microwave sub-peak. These electrons are assumed to be more abundant at higher energies ( $>E_0$ ; say,  $E_0 = 500$  to  $\sim 1,000$  keV) and less abundant at lower energies ( $<E_0$ ) than those electrons accelerated at the time of the first microwave sub-peak. As a consequence, the hard X-ray emission measured at  $\sim 100$  keV ( $\ll E_0$ ) is weaker whereas the microwave (17 GHz) emission remains prominent since the latter is caused mainly by more energetic electrons ( $\sim E_0$ ). (2) Electrons accelerated at the time of the first microwave sub-peak stream into a dense region where hard X-ray emissions are produced, whereas most of the electrons which are accelerated simultaneously with ions at the time of the second microwave sub-peak do not penetrate



**Fig. 1** Time profiles of microwave (a, 17 GHz), hard X-ray (b, 40–140 keV; c, 300–350 keV) and the prompt  $\gamma$ -ray line (d, 4.1–6.4 MeV) emissions for the 7 June 1980 event. Both the hard X-ray and prompt  $\gamma$ -ray line emissions were observed with the SMM  $\gamma$ -ray spectrometer with  $\sim 1$  (b),  $\sim 1$  (c) and  $\sim 2$  (d) s time resolutions. The microwave emission was observed with the 17-GHz polarimeter at Nobeyama with the  $e$ -folding time constant of 0.3 s (a).



**Fig. 2** Time profiles of microwave (a, 17 GHz), hard X-ray (b, 40–140 keV) and  $\gamma$ -ray (c, 4.1–6.4 MeV) emissions for the 21 June 1980 event. The time profiles of the microwave, hard X-ray,  $\gamma$ -ray and emissions are drawn with 0.3 (a)  $\sim 1$ , (b) and  $\sim 2$  (c) s resolutions.

to the dense region but give rise to hard X-ray emissions in a less dense region.

Our observations strongly suggest that two different kinds of acceleration work successively in the impulsive phase. If we neglect the possibility of explaining the observations by the injection property, our observations are not inconsistent with the two-step acceleration model recently proposed<sup>8,9</sup>, in which electrons and ions accelerated at the first step are further accelerated at the second step. However, another explanation, that the two kinds of acceleration are the two variants of one acceleration mechanism, is equally possible. When the acceleration mechanism works successively in a few seconds, the energization of particles in the second acceleration is affected by the product of the first acceleration and consequently produces a different energy and pitch-angle distribution of energetic particles with a different ion-to-electron ratio from that of the first acceleration. In fact, there is not much difference of the observed ion-to-electron ratio between the first and second microwave sub-peaks.

Other mechanisms proposed by various authors concerning the time lag of the  $\gamma$ -ray peaks with respect to the hard X-ray peaks try to explain the time lag in terms of the difference of either the transit time or the relaxation time differences between electrons and ions<sup>3-10</sup>. For the latter case we have made a numerical simulation in which the  $\gamma$ -ray time profile is calculated from the hard X-ray time profile. The result is in a good agreement with the observation if the ambient electron density of  $5 \times 10^{12} \text{ cm}^{-3}$  is assumed. However, none of these interpretations can explain the coincidence between the second microwave sub-peak and the  $\gamma$ -ray peak which is found in the present observations, because the microwave emission requires the presence of high-energy electrons. Otherwise, the coincidence would be considered merely fortuitous. Further investigations are needed to determine which of these interpretations is correct.

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## Microwave spectroscopy of H<sub>2</sub>O in the stratosphere and mesosphere

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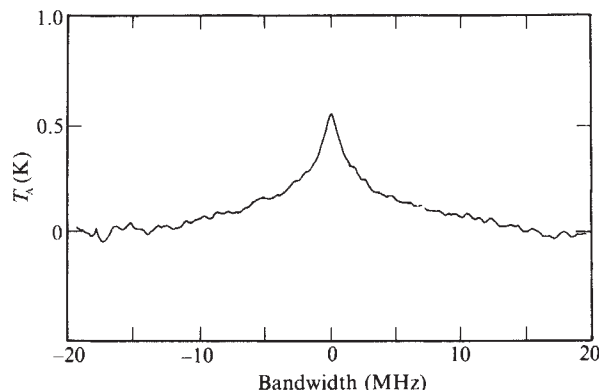
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**Ground-based microwave spectroscopy is a powerful technique for remote sensing of minor constituents, such as H<sub>2</sub>O, in the middle atmosphere that might otherwise be difficult to measure quantitatively. The 6<sub>16</sub>-5<sub>23</sub> H<sub>2</sub>O transition at 22.2 GHz is particularly well suited for this application. It is optically thin and at a low frequency which allows straight-forward inversion of spectra to recover altitude profiles up to**

**~80 km altitude. Because of considerable radioastronomical interest in this transition, which is non-thermally excited in the interstellar medium, many suitable receiver systems are available<sup>1-3</sup>. Monitoring of the H<sub>2</sub>O altitude profile on short and long time scales is practical and has been going on from Haystack Observatory for several years<sup>2,4,5</sup>. The principal limitation of this experiment is not receiver sensitivity—a few hours of integration are sufficient to produce an H<sub>2</sub>O atmospheric emission spectrum suitable for inversion—but, rather, baseline curvature in the receiver-spectrometer system. Baseline effects have limited the effective bandwidth of the Haystack experiments to 6.67 MHz making the effective lower altitude limit of profile inversion ~50 km. They are also a dominant source of error in recovered profiles in the 50–60 km range. We present here the first results of observations with a new receiver system developed specifically to observe atmospheric H<sub>2</sub>O which allowed a spectrometer bandwidth of 40 MHz to be used and, thus, allowed the H<sub>2</sub>O profile from ~35 to 80 km to be recovered. The resulting profile, although only a 'snapshot' of a probably time-dependent phenomenon, is consistent with previous measurements in the mesosphere and stratosphere.**

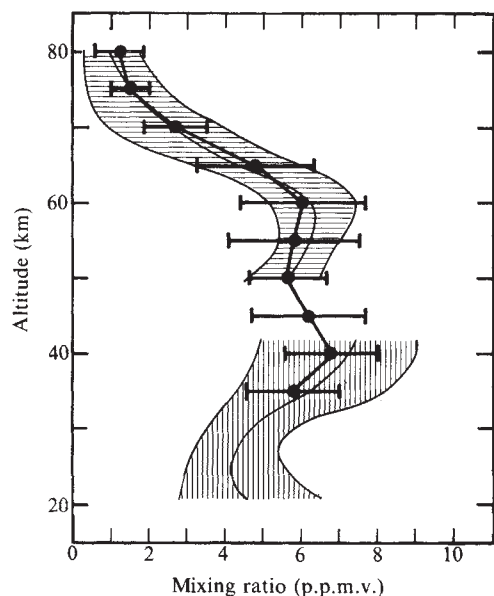
The H<sub>2</sub>O atmospheric emission observations were made on 24 March 1983 from Haystack Observatory in Westford, Massachusetts (lat. 42°37'). A new maser preamplifier developed for the Pennsylvania State University (PSU) by Haystack Observatory was substituted for the radioastronomy maser in the Naval Research Laboratory (NRL)-PSU aeronomy receiver system. Except for minor waveguide 'plumbing' changes to accommodate a different Dewar, the system and observing technique were physically the same as has been used for the past 3 yr (ref. 2). The new maser preamplifier, because it was optimized for the H<sub>2</sub>O frequency, had a wider and flatter r.f. bandpass. It also used a solid-state r.f. pump rather than the backward wave oscillator tube used on the radioastronomy maser and was, consequently, more gain stable. The spectrum, obtained with an autocorrelation spectrometer operating at 40 MHz total bandwidth (391-kHz effective resolution) after 110 min of integration, is shown in Fig. 1. No obvious pathological baseline curvature is present in the spectrum other than a higher noise level near the edges due to roll-off of the receiver bandpass.

The H<sub>2</sub>O emission spectrum was calibrated and inverted to produce the altitude profile shown in Fig. 2 using the same analysis as in previous experiments except that weighting functions out to  $\pm 15$  MHz from line centre were included<sup>5</sup>. Only limited information above ~70 km is contained in the spectrum because of the low spectral resolution. To improve the inversion, data taken for ~20 h before and ~10 h after at 13-MHz bandwidth (32-kHz resolution) were inverted to obtain an average profile for 23–25 March and this profile was used as an initial

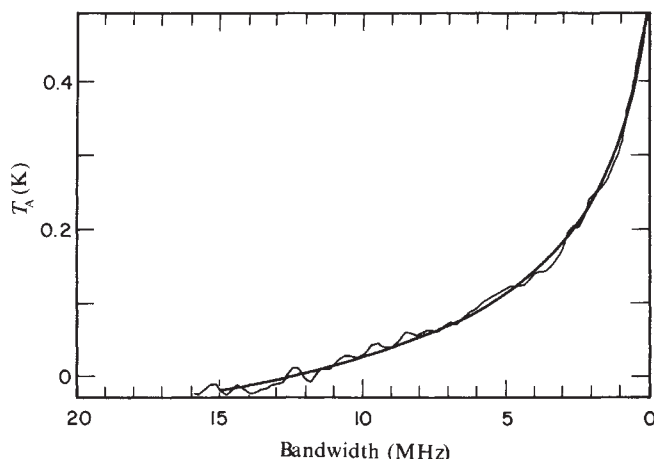


**Fig. 1** H<sub>2</sub>O emission spectrum at 12° elevation angle on 24 March 1983 at Haystack Observatory in Westford, Massachusetts. The total bandwidth was 40 MHz, spectral resolution was 391 kHz, and integration time was 110 min. The local surface weather was very good: clear sky and surface temperature of 1 °C. The glitches at the low frequency edge of the spectrum are due to roll-off of the receiver-spectrometer bandpass.





**Fig. 2** H<sub>2</sub>O altitude profile recovered from the data in Fig. 1 (heavy line) compared with the range of mesospheric profiles in 1980–82 in the NRL–PSU experiment<sup>5</sup> (horizontal hatching) and the average stratospheric profile from LIMS data for April 1979 (vertical hatching). Above ~70 km, a significant amount of information from 13-MHz bandwidth data taken before and after the 40-MHz bandwidth observations is implicitly included in the result.



**Fig. 3** The symmetrized and truncated version of data from Fig. 1 compared with a synthetic emission spectrum computed from the profile in Fig. 2.

condition of the inversion. The results above ~70 km are, thus, averages over longer than the 110 min period of wide band observation. In Fig. 3 a symmetrized and truncated version of the spectrum is compared with an emission spectrum computed from the inverted profile to illustrate the quality of the inversion. For comparison, average profiles above 50 km, from our previous work, and below ~50 km from the Nimbus-7 LIMS (Limb Infrared Monitor of the Stratosphere) experiment<sup>6</sup> are also plotted on Fig. 2. The LIMS data are typical of spring mid-latitude H<sub>2</sub>O profiles in the stratosphere obtained by both modern *in situ* and remote sensing techniques<sup>7</sup>. Although the comparison is between averaged profiles over different times and a short duration measurement, Fig. 2 may be a representative picture of the behaviour of H<sub>2</sub>O in the mid-latitude upper stratosphere and mesosphere. The H<sub>2</sub>O profile is characterized by a constant or slowly increasing with altitude mixing ratio in the stratosphere with, ultimately, a maximum value of 5–8 p.p.m.v. near 60 km and then a sharp decrease to a value of ≤2 p.p.m.v. above 75 km.

In conclusion, we emphasize several points concerning this measurement of the H<sub>2</sub>O profile in the upper stratosphere and

mesosphere. (1) The observed profile is approximately in agreement with our preconceptions about H<sub>2</sub>O in the stratosphere but, as noted in our previous observations, the H<sub>2</sub>O gradient above ~70 km is steeper than predicted using currently popular values of the eddy diffusion coefficient,  $K_z$ , in the mesosphere<sup>5</sup>. (2) In the sense of a comparison of a single measurement with longer term averages, our observations demonstrate that H<sub>2</sub>O profiles derived from microwave spectroscopy are consistent and continuous with profiles derived from other methods such as LIMS. (3) However, mesospheric H<sub>2</sub>O is variable on time scales as short as days so that detailed comparisons between various results should be made carefully and sceptically<sup>4</sup>.

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## Fe–Ni–S–O layer phase in C2M carbonaceous chondrites—a hydrous sulphide?

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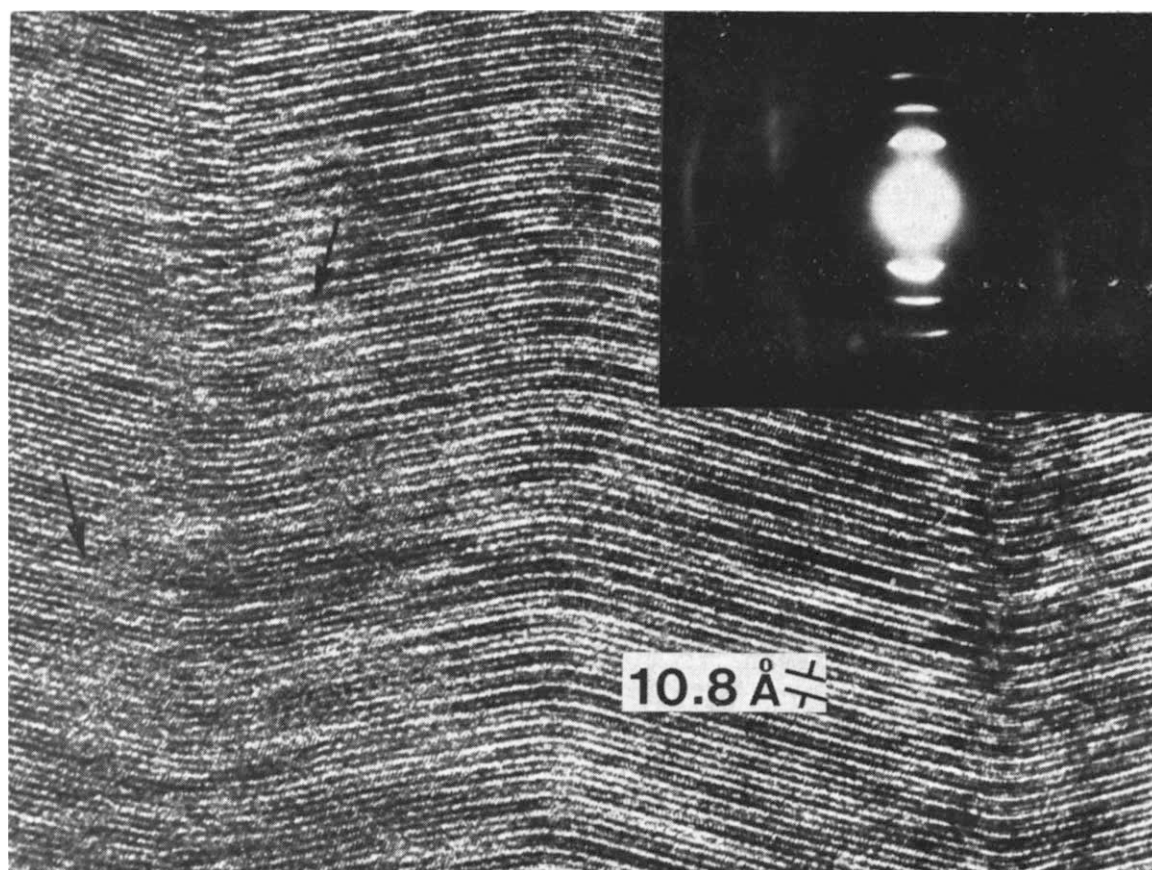
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It has been suggested<sup>1,2</sup> that the matrix minerals of type C2M carbonaceous meteorites are the products of aqueous alteration in a parent-body regolith. Phases occur within the matrix which differ from those commonly found in cases of terrestrial aqueous alteration and provide clues to the history of the meteorites. Poorly-characterized phases (PCP)<sup>3</sup> containing Fe, Ni, S, O and sometimes Cr, were first noted in the Murchison meteorite. Barber<sup>4</sup> found that amongst PCP there was a layer phase, rich in Fe, Ni, S and thought to contain oxygen, commonly having a fibrous or platy morphology, with a layer spacing of approximately 10.8 and 5.4 Å. We report here new data on this phase, obtained by high resolution electron microscopy, dispersive X-ray spectrometry and electron energy loss spectrometry, and we conclude that it is probably a hydrous sulphide.

The phase which we have studied (one of several embraced by the PCP label) is easily recognized in the transmission electron microscope (TEM) by means of its characteristic electron diffraction pattern and, to a lesser degree, by its morphology. It occurs infrequently as small, submillimetre-sized grains which appear by optical transmission microscopy of ultrathin sections as pale green-grey patches, often closely associated with phyllosilicates, as noted by Fuchs *et al.*<sup>3</sup>. We have found the phase in the meteorites Murchison, Cold Bokkeveld and Nawapali. Undoubtedly it also occurs in other meteorites, such as Mighei<sup>5</sup>. Bunch and Chang<sup>2</sup> have reported that Nogoya is particularly rich in PCP of various types, but we have so far failed to locate the particular phase discussed here.

Lattice images of the phase, obtained with the Cambridge 600 kV high resolution microscope (HVHREM)<sup>6</sup>, reveal undulatory sets of lattice planes, as shown in Fig. 1, corresponding to the 10.8 Å and 5.4 Å diffraction data reported earlier<sup>4</sup>. Between the regions of strong bending, the 10.8 Å lattice spacing is often very dominant over the 5.4 Å spacing, suggesting a double-layered structure. The HVHREM enables



**Fig. 1** HVHREM lattice image and corresponding electron diffraction pattern from region of undulatory PCP layer phase in the Murchison meteorite, exhibiting dominant 10.8 Å spacings, with subsidiary 5.4 Å layers and 7.7 Å transverse spacings (arrowed).

cross grating patterns to be seen in some regions but over most of the images there is random stacking of the doubled layer, a feature also observed in the layer stacking of some phyllosilicates (for example ref. 7). This turbostratic structure is characteristic of and best known in smectites<sup>8</sup>, where the term implies that there is no three-dimensional lattice as a consequence of pervasive stacking disorder. The apparent periodicities and regions of disorder were further evaluated by optical diffractogram analysis of small areas of the HVHREM negatives. This approach indicated that interplanar spacings of 7.7, 2.7, 2.4, 2.25 and 2.1 Å were present in the more ordered volumes of the phase, additional and transverse to the 10.8 Å and 5.4 Å layer spacings. These spacings were calibrated in terms of carbonate lattice images<sup>9</sup> recorded at the same magnification. Moreover, electron diffraction patterns from tilted crystals indicated additional interplanar spacings of 3.6, 3.5, 2.9 and 1.65 Å. The common layer-to-layer disorder was confirmed by linear streaks in the diffractograms, transverse to the diffraction spots for the layer rows and most strongly through the reflections corresponding to the 5.4 Å spacing, as shown in Fig. 2.

The lattice distortions associated with the layer plane inflections and the extent of disorder are too severe for convergent beam electron diffraction methods<sup>10</sup> (for which we used a Philips 400T TEM) to give useful information about the space group of the phase. Nonetheless, we believe that the material is one

phase, even though occasional intergrowths of different layer spacing have been found to occur. The phase grows in close proximity to the Fe, Mg platy phyllosilicates in the matrix (which are analogous to terrestrial lizardites and cronstedtite) but, as shown in Fig. 3, amorphous transition layers normally separate the new phase from phyllosilicates. Occasionally intimate interlayering occurs, with parallelism between the layer planes, a feature also noted by Tomeoka and Buseck<sup>5</sup>.

Chemical analysis of the phase has been carried out by energy dispersive X-ray spectrometry (EDS) for the heavier elements and by electron energy loss spectrometry (EELS) for oxygen.

Using troilite (FeS) from the Bella Roca meteorite as a thin foil reference standard for EDS, we measured Fe/S values on three fairly homogeneous patches of the phase in the Murchison meteorite. The values averaged to give  $1.39 \pm 0.08$  (earlier results<sup>4</sup> obtained, using an older electron microscope and without a sulphide standard, were erroneous, apparently because of loss of sulphur during analysis). Ni/Fe values were found to vary between patches of the phase. As Ni and Fe are close together in the Periodic Table, their ratios were evaluated without use of standards, relying on a previously-determined detector scaling factor<sup>11</sup> for these elements, which gave Ni/Fe ratios between 0.03 and 0.04.

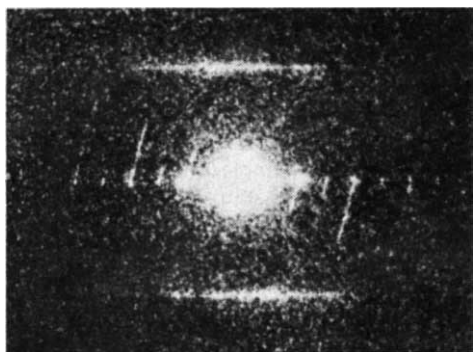
Fe/O ratios were deduced from the sizes of respective L and K absorption edges in EELS (Fig. 4) using a  $\pi/2$  sector magnet

**Table 1** EELS data for PCP spectrum shown in Fig. 4 and for a spectrum from haematite thin foil standard

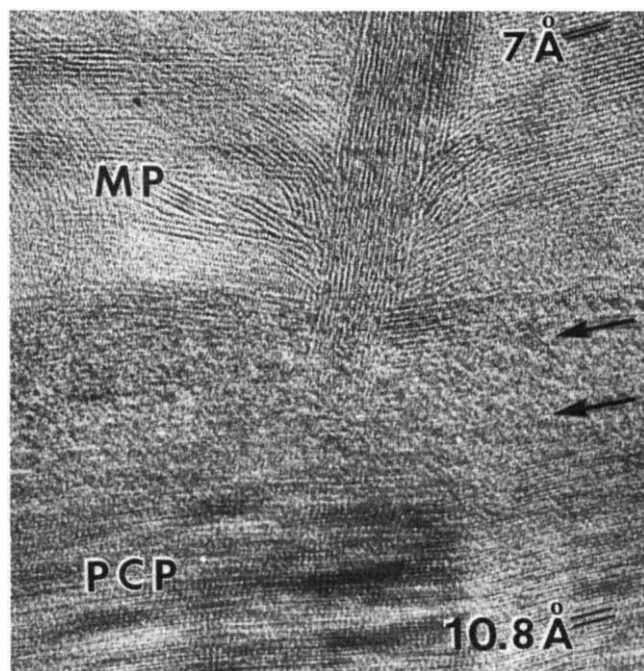
|                                          | Fe L edge                                          |                                                | O K edge                                           |                                                | Atomic Fe/O ratio normalized by respective $\sigma$ |
|------------------------------------------|----------------------------------------------------|------------------------------------------------|----------------------------------------------------|------------------------------------------------|-----------------------------------------------------|
|                                          | Counts in 100 eV window after $E^{-r}$ subtraction | Calculated $\sigma$ (cm <sup>2</sup> per atom) | Counts in 100 eV window after $E^{-r}$ subtraction | Calculated $\sigma$ (cm <sup>2</sup> per atom) |                                                     |
| PCP                                      | 144,220                                            | $0.230 \times 10^{-20}$                        | 132,308                                            | $0.194 \times 10^{-20}$                        | 0.92                                                |
| Haematite Fe <sub>2</sub> O <sub>3</sub> | 11,600                                             | $0.230 \times 10^{-20}$                        | 11,664                                             | $0.194 \times 10^{-20}$                        | 0.84                                                |

$\sigma$  = partial cross-section;  $E$  = energy loss;  $r$ , a fitted constant.

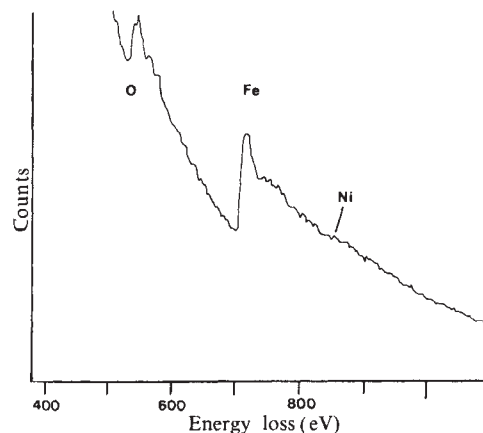




**Fig. 2** Optical diffractogram from part of HVHREM image showing linear streaking through 10.8 and 5.4 Å diffraction spots, indicating random stacking of the principal atom layers.



**Fig. 3** HVHREM lattice image of contact region between PCP layer phase and matrix phyllosilicates (MP) in the Murchison meteorite. Note band of partially amorphous material between arrows.



**Fig. 4** Part of EELS spectrum of PCP phase showing the K edge of oxygen and L edges of iron and nickel, measured using 100 kV incident electrons and 16 mrad collection semi-angle.

representing an error consistent with the general experience of users of EELS (see ref. 16, where slightly smaller errors were found (~10%), but in edges of similar L-type).

These results provide the chemical formula for the phase as  $\text{Fe}_{1.4}\text{S O}_{1.3}$ . Extensive searches of the literature and the powder diffraction files of the Joint Committee on Powder Diffraction Standards show that the electron diffraction data/lattice spacings and derived formula correspond to no known sulphate<sup>17,18</sup> or oxy-salt. The aqueous alteration origin of the matrix minerals, the disordered layer stackings observed, and the intimate relationship between Fe, Mg phyllosilicates and the PCP material raises the possibility of a hydrous phase. Some support for this idea comes from the limited data of Jambor<sup>19</sup> on an oxygen-bearing iron sulphide found in the Muskox intrusion, North-West Territories. Jambor noted that the mineral, which occurred as millimetre-sized veins in proximity to serpentines and magnetite (as it does in the Murchison matrix) appeared black. This does not conflict with the properties of the matrix phase, in our view, since meteorite matrix is black in thick sections. Jambor's fibrous mineral gave X-ray diffraction spacings of 10.9, 5.45, 1.83 and 1.55 Å, very similar to those of the PCP. He suggested a formula  $3\text{FeS}_2(\text{Mg}, \text{Fe}^{3+})(\text{OH})_{2+n}$  for the mineral (where  $n$  is additional hydroxyl to balance ferric iron), a formula compatible with our data.

A more precise characterization of the phase is dependent on improvements in quantification of the EELS method and finding a larger mass of well-ordered homogeneous phase in a meteorite matrix. Meanwhile, our view is that the Fe-Ni-S-O layer phase in PCP is probably a hydrous sulphide, whose hydroxyl layers tend to grow parallel to and can be epitactic with the layer planes in serpentines.

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attached to a Philips EM400 electron microscope and a Link Systems computer. The collection semi-angle was 16 mrad and the specimen thickness in the regions analysed less than about two extinction distances. After  $E^{-r}$  background subtraction (where  $E$  represents energy-loss and  $r$  is a fitted constant), the edges were integrated over 100-eV windows and the integrals normalized against calculated partial cross-sections<sup>12,13</sup>. The resulting Fe/O ratio was 0.92 (see Table 1) with an error which is estimated with difficulty for the following reason.

Although EELS yields reliable qualitative information, current quantitative procedures, which require defined collection angles after electron scattering, do not take proper account of multiple elastic-inelastic scattering<sup>14</sup>. This complicates the angular distribution of inelastically scattered electrons, which depends on specimen thickness. These complications may be expected to cause larger errors than those arising from approximations used in calculated partial cross-sections, which give consistent values when obtained in a variety of ways, whether using hydrogenic models or Hartree-Slater wavefunctions (see ref. 15). An example of the errors involved was obtained by measuring the ratio of Fe/O edge intensities in a haematite standard (Table 1). If these measured partial cross-sections were to be used instead of the calculated ones, the resulting Fe/O ratio in the PCP phase would be 21% less than that given above,

# Rare gases from the undepleted mantle?

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If the concept of a depleted/undepleted mantle system is valid, rare gas isotopic composition and abundances should be different in continental and plume areas fed from the undepleted mantle than in oceanic glasses fed from a depleted mantle<sup>1</sup>. Ultramafic inclusions in basalt have been studied in attempts to find examples of undepleted mantle trapped gases<sup>2-6</sup>. In particular, Kaneoka and Takaoka measured the helium and argon isotopic abundances in olivine nodules and phenocrysts from Hawaiian basalt<sup>7</sup>. The nodules are characterized by mid-ocean ridge basalt (MORB)-type  $^{40}\text{Ar}/^{36}\text{Ar}$  and  $^3\text{He}/^4\text{He}$  ratios, while the phenocryst data show lower  $^{40}\text{Ar}/^{36}\text{Ar}$  and higher  $^3\text{He}/^4\text{He}$ ; these latter data were interpreted to indicate an undepleted mantle source relative to that for the nodules and oceanic tholeiites. I have measured  $^4\text{He}$ ,  $^{40}\text{Ar}$  and  $^{36}\text{Ar}$  abundances in two Hawaiian ultramafic nodules, and find clear evidence of incomplete retention of mantle gases plus significant atmospheric contamination. Comparison with the previously measured phenocrysts indicates the prevalence of these complications in all such ultramafic samples. Conclusions concerning the nature of the rare gases in the undepleted mantle based on such samples are invalid.

If a suite of samples has trapped argon from a given reservoir and quantitatively retained it while adding varying amounts of atmospheric contamination, the total  $^{40}\text{Ar}/^{36}\text{Ar}$  in each of the samples will fit the relationship<sup>8</sup>:

$$\frac{^{40}\text{Ar}}{^{36}\text{Ar}} = \frac{(m-a)^{40}\text{Ar}_m}{(m)^{36}\text{Ar}} + a \quad (1)$$

where  $a$  is the value of the atmospheric  $^{40}\text{Ar}/^{36}\text{Ar}$  ratio (295.5),  $m$  is the trapped (mantle) ratio value (unknown), and  $^{40}\text{Ar}_m$  is the abundance of trapped (mantle)  $^{40}\text{Ar}$ . Plots of  $^{40}\text{Ar}/^{36}\text{Ar}$  against  $(^{36}\text{Ar})^{-1}$  have demonstrated the linear relationship required by equation (1) for several oceanic tholeiites<sup>9,10</sup>, but previous investigators of ultramafic samples generally have contented themselves with making single measurements on individual samples; from such data it is impossible to disentangle the atmospheric contamination. Recently, Rison and Kyser published Ar data from five samples of a Hawaiian dunite and found  $^{40}\text{Ar}_m$  ranging from  $48$  to  $68 \times 10^{-8} \text{ cm}^3 \text{ g}^{-1} \text{ STP}$ ; three samples of another Hawaiian dunite as measured by the Berkeley and Missouri groups gave  $^{40}\text{Ar}_m = 150, 172$  and  $214 \times 10^{-8} \text{ cm}^3 \text{ g}^{-1} \text{ STP}$  (ref. 11). The data were interpreted as indicating a single mantle component mixed with atmospheric argon, although the straight line of equation (1) for the former samples barely intercepts the rather liberal error bars assigned and the line needed to fit the latter data extrapolates to an atmospheric argon  $^{40}\text{Ar}/^{36}\text{Ar}$  ratio of  $-2,922$ . Such a ratio is clearly nonsensical and indicates the need for further investigation. I have measured these isotopes in two Hawaiian olivine nodules; the data are presented in Table 1 and Figs 1 and 2. Nodule XB was composed of olivine: gram-sized samples were prepared of crystals  $\geq 1 \text{ mm}$ , each sample from varying locations in the nodule. The data (Fig. 1) show the general trend expected of a mixture of trapped and atmospheric Ar, but are not fitted by a straight line, indicating that the trapped component was not quantitatively retained. This result is not surprising in retrospect; in contrast to the situation for oceanic basalts, where Ar dissolved in the magma is transported to the surface and then instantaneously trapped under high hydrostatic pressure in a rapidly chilled glass skin, the argon in the nodule has ample opportunity for high-temperature diffusion out into the magma

both during transport to the surface and during the subsequent slow cooling of the host basalt.

Several aliquots were prepared from nodule 67TES, which was composed of roughly equivalent amounts of olivine and augite crystals. Samples 1, 2 and 4 were taken from the outer portion of the nodule; samples 1 and 2 were  $\geq 1 \text{ mm}$  crystals, sample 4 was  $0.5\text{--}1 \text{ mm}$ . Samples 3, 5 and 6 were taken from the interior, crystal size  $\geq 1 \text{ mm}$ . Sample 7 consisted of pure augite crystals,  $\geq 1 \text{ mm}$ . The data (Fig. 2) fall on straight lines through the atmospheric point, each line corresponding to the nature of the aliquot. Samples 3, 5 and 6 (interior portion) define a line corresponding to the retention of  $205 \times 10^{-8} \text{ cm}^3 \text{ g}^{-1} ^{40}\text{Ar}_m$ , with varying amounts of atmospheric contamination. Samples 1 and 2, from the outer portion, retained only  $153 \times 10^{-8} \text{ cm}^3 \text{ g}^{-1}$ , and sample 4 (the smaller crystals) retained only  $78 \times 10^{-8} \text{ cm}^3 \text{ g}^{-1}$ . The augite crystals retained the most  $^{40}\text{Ar}_m$ ,  $300 \times 10^{-8} \text{ cm}^3 \text{ g}^{-1}$ . These results are reflected also in the  $^4\text{He}$  data. There is no one sample or set of samples which can be accepted as having trapped and retained an argon or helium component representative of the source region. Rather, the pattern of depletion of  $^{40}\text{Ar}_m$  is that which would be expected from a system undergoing high-temperature diffusion loss of argon incorporated in the crystals at their time of formation. The olivine nodule data of Kaneoka and Takaoka lie within the same envelope (Fig. 2): it is likely that they illustrate the same situation. The question is, do the phenocryst data of Kaneoka and Takaoka represent a similar argon environment in the pertinent source region, modified by subsequent atmospheric contamination and diffusive loss, or do they represent a different (that is undepleted) argon regime? The demonstrated occurrence of loss and addition processes in the nodules certainly portends their occurrence in the smaller phenocrysts: the iden-

**Table 1** Rare gas contents of Hawaiian ultramafic nodules

| No. | Sample                       | $^4\text{He}$ | $^{40}\text{Ar}$ | $^{36}\text{Ar}$ | $^{36}\text{Ar}$ |
|-----|------------------------------|---------------|------------------|------------------|------------------|
| 1   | 67B <sub>1-2</sub>           | 38.6          | 152              | 0.0674           | 2,256            |
| 2   | 67B <sub>2-3, 4</sub>        |               |                  |                  |                  |
|     | L                            | 1             | 20.8             | 0.059            | 353              |
|     | H                            | 30            | 135              | 0.039            | 3,450            |
|     | Total                        | 31            | 156              | 0.098            | 1,590            |
| 3   | 67B <sub>3,5-5, 9, 10</sub>  |               |                  |                  |                  |
|     | L                            | —             | 10               | 0.044            | 329              |
|     | H                            | 35            | 193              | 0.0264           | 7,300            |
|     | Total                        | 35            | 203              | 0.0704           | 2,833            |
| 4   | 67D <sub>1,2</sub>           |               |                  |                  |                  |
|     | L                            | —             | 8.3              | 0.0268           | 310              |
|     | H                            | 15            | 78.3             | 0.0199           | 3,940            |
|     | Total                        | 15            | 86.6             | 0.0467           | 1,852            |
| 5   | 67B                          |               |                  |                  |                  |
|     | L                            | —             | 10.3             | 0.035            | 295              |
|     | H                            | 36            | 222              | 0.0839           | 2,614            |
|     | Total                        | 36            | 232              | 0.120            | 1,851            |
| 6   | 67B <sub>4</sub>             |               |                  |                  |                  |
|     | L                            | —             | 6                | 0.0199           | 301              |
|     | H                            | 39            | 203              | 0.0326           | 6,227            |
|     | Total                        | 39            | 209              | 0.0525           | 3,981            |
| 7   | 67A                          | 48            | 307              | 0.152            | 2,020            |
| 8   | XB <sub>3-17</sub>           | 20            | 51.6             | 0.0414           | 1,246            |
| 9   | XB <sub>1-11, 12</sub>       |               |                  |                  |                  |
|     | L                            | 0.5           | 8.5              | 0.024            | 354              |
|     | H                            | 6.0           | 49               | 0.00994          | 4,930            |
|     | Total                        | 6.5           | 57.5             | 0.03394          | 1,705            |
| 10  | XB <sub>3-16, 18</sub>       |               |                  |                  |                  |
|     | L                            | 0.7           | 8.6              | 0.0256           | 336              |
|     | H                            | 30.6          | 73               | 0.0131           | 5,572            |
|     | Total                        | 31            | 82               | 0.039            | 2,100            |
| 11  | XB <sub>2,5-14, 15, 23</sub> |               |                  |                  |                  |
|     | L                            | 1             | 12               | 0.039            | 308              |
|     | H                            | 36            | 99.3             | 0.0197           | 5,030            |
|     | Total                        | 37            | 111              | 0.0587           | 1,890            |

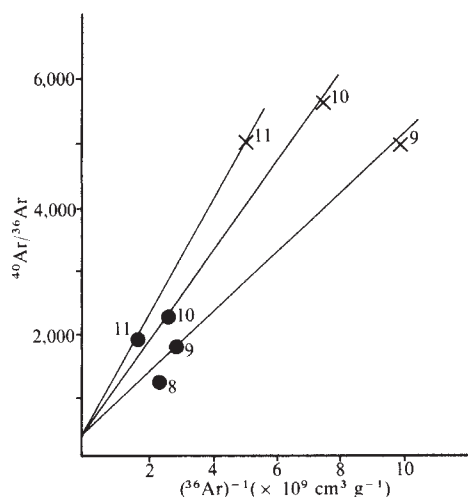
Units,  $10^{-8} \text{ cm}^3 \text{ g}^{-1} \text{ STP}$ . A denotes augite crystals, B denotes crystals  $\geq 1 \text{ mm}$ , D denotes crystals  $0.5\text{--}1 \text{ mm}$ . L and H denote low- and high-temperature release patterns, respectively.



**Table 2** He and Ar summaries

| Sample               | $^3\text{He}$<br>( $\times 10^{-10}$ ) | $^4\text{He}_r$<br>( $\times 10^{-5}$ ) | $^{36}\text{Ar}$<br>( $\times 10^{-10}$ ) | $^{40}\text{Ar}_r$<br>( $\times 10^{-8}$ ) |
|----------------------|----------------------------------------|-----------------------------------------|-------------------------------------------|--------------------------------------------|
| Oceanic tholeiites   | 0.5–3<br>(13, 14)                      | 0.1–2.5<br>(13–15)                      | 1–7<br>(10, 15)                           | 30–310<br>(10, 15)                         |
| Hawaiian nodules     | 0.03–0.15<br>(7)                       | 0.006–0.13<br>(7, this work)            | 2–14<br>(7, this work)                    | 50–300<br>(7, this work)                   |
| Hawaiian phenocrysts | 0.005–0.015<br>(7)                     | 0.002–0.003<br>(7)                      | 10–22<br>(7)                              | 1.6–6<br>(7)                               |

Units,  $\text{cm}^3 \text{g}^{-1}$  STP. References are shown in parentheses.

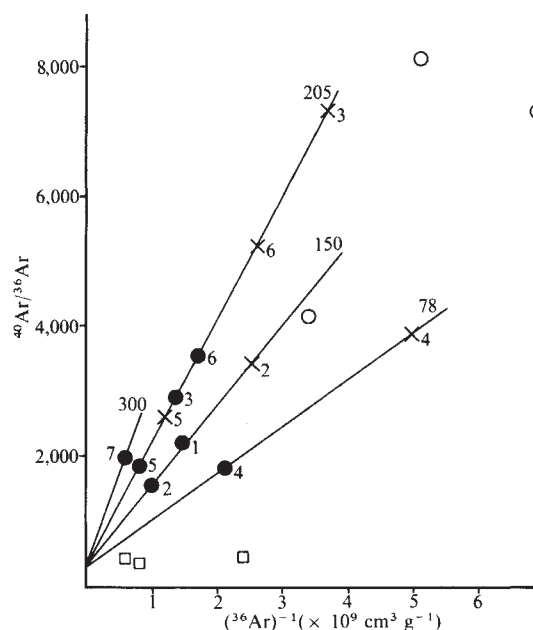


**Fig. 1**  $^{40}\text{Ar}/^{36}\text{Ar}$  versus  $(^{36}\text{Ar})^{-1}$  for olivine nodule XB.  $\times$ , The high-temperature release data;  $\bullet$ , the total abundances.

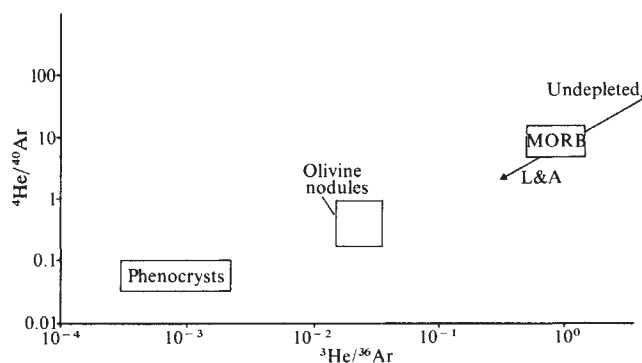
tical high-temperature environment coupled with greater surface/volume ratio should lead to even greater effects of both processes. Indeed we see this expectation reflected in the data.

Consider the effects of each process: diffusive loss does not materially affect the  $^{40}\text{Ar}/^{36}\text{Ar}$  ratio, but depletes the sample in both isotopes. Atmospheric contamination lowers the initially high trapped ratio by enriching both isotopes at a constant ratio of 295.5. The effect of the latter process is to slide the data down within the envelope of straight lines shown in Fig. 2 towards the y intercept; the effect of the former is to shift the data horizontally to the right. This is precisely what is seen in the phenocryst data relative to the nodules (Fig. 2).

Let us next investigate the effect on the helium isotopes, by considering the ratios of the primordial isotopes  $^3\text{He}/^{36}\text{Ar}$  and the radiogenic isotopes  $^4\text{He}/^{40}\text{Ar}$ . Oceanic tholeiite glasses show remarkably similar ratios of  $^3\text{He}/^4\text{He} \sim 10^{-5}$ ,  $^{40}\text{Ar}/^{36}\text{Ar} \sim 10^4$ , and  $^4\text{He}/^{40}\text{Ar} \sim 15 \pm 5$ . Lower values of  $^{40}\text{Ar}/^{36}\text{Ar}$  have been shown to be due to atmospheric contamination while lower values of  $^4\text{He}/^{40}\text{Ar}$  are due to  $^4\text{He}$  loss by post-magmatic diffusion<sup>10</sup>. Samples with high  $^4\text{He}/^{40}\text{Ar}$  have a  $^4\text{He}/^{136}\text{Xe}_r$  ratio nearly an order of magnitude higher than the radiogenic production ratio<sup>12</sup>, due to mass-fractionation enrichment of the lighter element. Therefore, an enrichment of the values of  $^4\text{He}/^{40}\text{Ar}$  and  $^3\text{He}/^{36}\text{Ar}$  by a factor  $\leq 3$  might be expected, so that the mantle source of the tholeiites has  $^3\text{He}/^{36}\text{Ar} \sim 0.5$ – $1.5$  and  $^4\text{He}/^{40}\text{Ar} \sim 5$ – $15$ . The lower limit of this latter value is about that expected for closed system radiometric production in a mantle with  $\text{K}/\text{U} \sim 10^4$ . If these ratios represent the depleted mantle, what values would be expected for the undepleted mantle? As any process of volatile depletion would tend to remove the lighter, more volatile gases more efficiently, the undepleted mantle must be characterized by higher  $^3\text{He}/^{36}\text{Ar}$  and  $^4\text{He}/^{40}\text{Ar}$ . On the other hand, post-magmatic diffusive loss of mantle gases would involve helium loss proceeding more rapidly than argon, and addition of atmospheric gases must involve greater addition of argon than helium both because of the atmospheric ratio  $\text{Ar}/\text{He} = 1,788$  and because argon is more



**Fig. 2** Olivine-augite nodule 67TES.  $\times$ , The high-temperature release data. The data of Kaneoka and Takaoka are shown as open circles (nodules) and squares (phenocrysts). The numbers at the top of each line give the amounts of trapped  $^{40}\text{Ar}_m$  in units of  $10^{-8} \text{cm}^3 \text{g}^{-1}$ .



**Fig. 3** He/Ar: summaries of the radiogenic versus primordial isotopes for oceanic glasses (MORB), nodules and phenocrysts. The arrows show the direction of undepleted mantle, or gases affected by loss and atmospheric contamination (L & A), relative to MORB.

easily surface-adsorbed than helium; the effect of both these processes is to lower both the  $^3\text{He}/^{36}\text{Ar}$  and  $^4\text{He}/^{40}\text{Ar}$  ratios. Thus, if the phenocrysts represent undisturbed undepleted mantle they must lie above and to the right of the oceanic tholeiites in a  $^3\text{He}/^{36}\text{Ar}$  versus  $^4\text{He}/^{40}\text{Ar}$  diagram, and if they represent a mantle source similar to the tholeiites but have been affected by loss of mantle gases and addition of atmospheric contamination they must lie below and to the left. The data are shown in Fig. 3. The conclusion is obvious. The relative position of the nodule and phenocryst samples is even more conclusive; if they are both to be considered representative of their mantle

source, then if the nodules came from a depleted mantle and the phenocrysts from undepleted mantle, their positions on the diagram would have been reversed.

Finally, the measured absolute abundances of all the isotopes support this notion. An undepleted mantle will have higher concentrations of the primordial isotopes  $^3\text{He}$  and  $^{36}\text{Ar}$ , due simply to the nature of the depletion; the undepleted mantle must also have higher concentrations of the radiogenic isotopes  $^4\text{He}$  and  $^{40}\text{Ar}$ , since not only volatiles but also incompatible elements such as U and K are depleted in the depleted mantle. The absolute concentrations of the helium and argon nuclides are given in Table 2. For the primordial isotope  $^3\text{He}$  and the radiogenic isotopes  $^4\text{He}$  and  $^{40}\text{Ar}$ , the phenocrysts have much lower abundances than either the nodules or the tholeiites, exactly opposite to what would be expected if the phenocrysts have quantitatively trapped these gases from the undepleted mantle. Only for  $^{36}\text{Ar}$  are they higher, and this is to be expected from atmospheric contamination.

These arguments fit together to give a coherent picture: the rare gases in the ultramafic samples, both phenocryst and nodule,

are not representative of their mantle source but have been affected by mass-dependent loss and addition processes. This was entirely expected, considering their emplacement, in contrast to the rapid chilling of oceanic tholeiitic glasses. The same suspicion applies to all ultramafic samples used for rare gas estimation of their mantle source regions. The helium ratio may have been largely unaffected by either process, both isotopes being lost to roughly the same extent and neither being enriched due to the low abundance of helium in the atmosphere and a low predisposition of helium towards surface adsorption processes, but the absolute He abundances in the ultramafic samples certainly do not reflect those in the respective mantle source regions. Argon in the ultramafic samples has been skewed both in absolute abundance and isotopic ratio. Until independent evidence is provided attesting to quantitative retention of mantle gases and minimal addition of atmospheric gases in samples which may have been supplied from an undepleted mantle, we are not justified in accepting any such data as indicative of the rare gases ambient in the undepleted mantle.

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## Metamorphic and tectonic evolution of granulites, Arunta Block, central Australia

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Petrographical and chemical evidence presented here indicate a prolonged metamorphic evolution of the granulite terrain in the northern Strangways Range, Arunta Block, central Australia (NSR in Fig. 1), beginning with migmatization, followed by dry granulite metamorphism at high temperatures ( $T$ ) and moderate pressures ( $P$ ) (estimated at  $850\text{--}920^\circ\text{C}$ ,  $8\pm 1\text{ kbar}$ ). This occurred at  $\sim 1,800\text{ Myr}$  (refs 2, 3), very shortly after deposition of the protolith<sup>4</sup>, a bimodal volcanic suite<sup>5</sup> and associated sediments. Granulite metamorphism was followed by isobaric cooling, during which there was an episode of pervasive but partial hydration. Later retrogression and hydration, localized along shear zones, occurred at the normal geothermal gradient in the kyanite stability field. A tensional, rift-like regime best describes the geological environment in which the protolith formed, was deeply buried, metamorphosed with high heat flow, and then remained in isostatic equilibrium. Metamorphic parageneses recording uplift may be matched with mid-Palaeozoic tectonic events in central Australia. If this correlation is correct, the rocks of the northern Strangways Range may have remained deeply buried for 1,400 Myr, during which the shear zones were reactivated several times (Fig. 2).

Granulites in the northern Strangways Range (Fig. 1) formed from a bimodal, predominantly felsic volcanic complex<sup>5</sup>, with minor chloritic tuffs<sup>6</sup> and calcareous sediments. The felsic units are part of a chemically distinctive suite of acid volcanics and granites, characterized by high  $\text{K}_2\text{O}$ , high  $\text{Al}_2\text{O}_3$ , and low  $\text{TiO}_2$  and  $\text{MgO}$  for a given  $\text{SiO}_2$  content; these were emplaced in the North Australian Orogenic Province at 1,880–1,800 Myr (refs 4, 7). The Rb–Sr date for the granulite metamorphism of  $1,820\pm 60\text{ Myr}$  and low initial  $^{87}\text{Sr}/^{86}\text{Sr}$  ratio of  $0.704\pm 0.003$  (ref. 3) indicates a very short period of crustal residence between deposition and metamorphism. By analogy with other mid-

Proterozoic domains in the North Australian Orogenic Province and elsewhere, these volcanics were emplaced in a tensional regime following a major mantle differentiation event at 2,100–1,900 Myr in which large volumes of material were accreted to the base of the crust<sup>4</sup> (Fig. 3a).

Extensive migmatization in the felsic rocks was followed by dry granulite metamorphism characterized *inter alia* by orthopyroxene–orthoclase assemblages (Table 1) which required very low water pressures<sup>8</sup>.  $\text{CO}_2$  flushing<sup>9</sup> seems probable because the migmatites remained *in situ*, and removal of  $\text{H}_2\text{O}$  in a melt phase did not occur. Estimated pressure–temperature conditions are  $8\pm 1\text{ kbar}$ ,  $850\text{--}920^\circ\text{C}$  for the western part of the area studied, and less extreme by 0.5 kbar,  $50\text{--}60^\circ\text{C}$  in the east, from the comparison of garnet–cordierite–orthopyroxene–quartz assemblages, coexisting pyroxenes, and clinopyroxene–plagioclase–quartz assemblages (Fig. 2).

The rocks then cooled isobarically from the abnormally hot conditions to a normal geothermal gradient in the kyanite field<sup>10</sup>. Early in this cooling there was a phase of pervasive but incomplete regional hydration in which biotite–phlogopite formed from orthopyroxene–orthoclase (Bi stage in Fig. 2, Table 1). In particular, in silica–undersaturated rocks, orthopyroxene–orthoclase–spinel hydrated to phlogopite–sapphirine or phlogopite–cordierite and orthopyroxene–orthoclase–sapphirine hydrated to phlogopite–cordierite. Such hydration reactions produced enclave or corona textures in which sapphirine or cordierite rims spinel and cordierite rims sapphirine<sup>11</sup>. Subsequently, cordierite and sapphirine, including sapphirine in the coronas, reacted to form fine-grained orthopyroxene–sillimanite and cordierite, particularly in garnet–orthopyroxene–cordierite–quartz assemblages, was replaced at its margins by orthopyroxene–sillimanite intergrowths<sup>11</sup>. Theoretical calculations indicate that both these cordierite reactions have low positive slopes in  $P$ – $T$  space<sup>12</sup>, thus indicating a near-isobaric cooling path.

The metamorphic stages that followed the near-isobaric cooling are recognized through hydrous assemblages developed along shear zones transecting the granulite terrain. The earliest of these (the Ky–Ged stage of Fig. 2) was marked by hydration of orthopyroxene to orthoamphiboles, and development of gedrite, kyanite and zirconian staurolite in erstwhile cordierite-bearing rocks from the eastern part of the area studied where estimated  $P$ – $T$  conditions were 7.5–8.7 kbar,  $650\text{--}720^\circ\text{C}$ . In



calc-silicate rocks from the western outcrops, rims of grossularite-rich garnet and quartz formed between wollastonite and scapolite, but the scapolite  $[(Ab_{15}An_{85})_3CaCO_3]$  approximately remained stable, indicating a minimum temperature of 750 °C (ref. 13). This stage is interpreted as encompassing one or more hydrations and deformations which occurred at the normal geothermal gradient. The Ky-Ged stage was followed by the formation of cordierite from quartz-kyanite-gedrite  $\pm$  staurolite (Cd-Ky-Ged stage, Fig. 2) enclosing enclaves of corroded kyanite and staurolite. This is interpreted as the result of an uplift of 3–4 km. Following uplift, renewed isobaric cooling began to restore conditions to the normal geothermal gradient (Sil-Str stage of Fig. 2) and sillimanite and staurolite began to form in cordierite of the cordierite-kyanite-gedrite stage. Finally quartz-chlorite assemblages were overprinted in the most intensely deformed parts of the shear zones.

This metamorphic evolution can be divided into three phases: the formation of the protolith, initial metamorphism and isobaric cooling; the formation of gedrite stage assemblages in the shear zones; and the final unroofing which began with the cordierite-kyanite-gedrite stage. Conventional tectonic models do not provide any totally satisfactory explanation of the tectonic environment in which such a metamorphic history could have taken place.

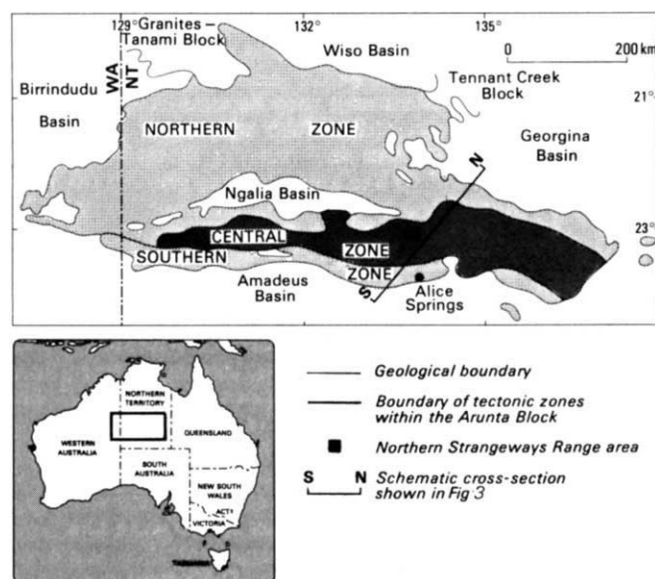


Fig. 1 The Arunta Block, central Australia, showing the major tectonic subdivisions, and the location of the schematic cross-sections shown in Fig. 3.

That the rocks were rapidly buried from surface conditions to depths of 25–30 km is indicated by the short time between deposition and metamorphism; but the subsequent near-isobaric cooling would have been dependent on prolonged isostatic equilibrium. Thus, the tectonic regime is considered more akin to that observed at modern passive margins where continent breakup has occurred<sup>14–16</sup> than to that of active plate margins where the pressure-temperature path of rocks deeply buried by overthrusting normally involves uplift while the rocks are still hot<sup>17</sup>. A rift-like environment accounts for many features of the early evolution of the Arunta Block. Bimodal volcanism is indicative of a tensional regime<sup>18,19</sup>. Down-warping and rapid sedimentation is a feature of rifts, but in the Phanerozoic, extreme extension allowing major down-warping

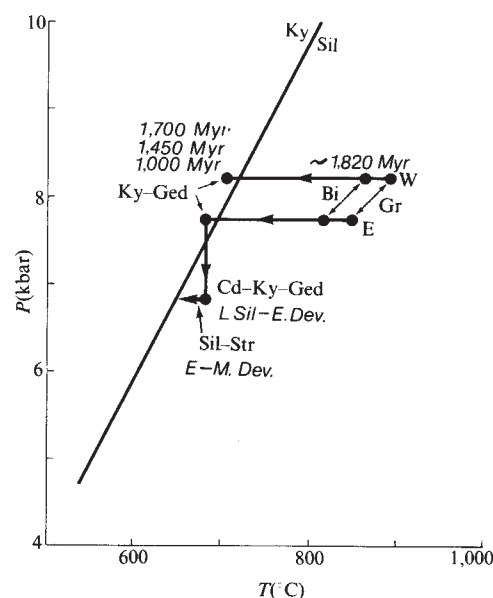
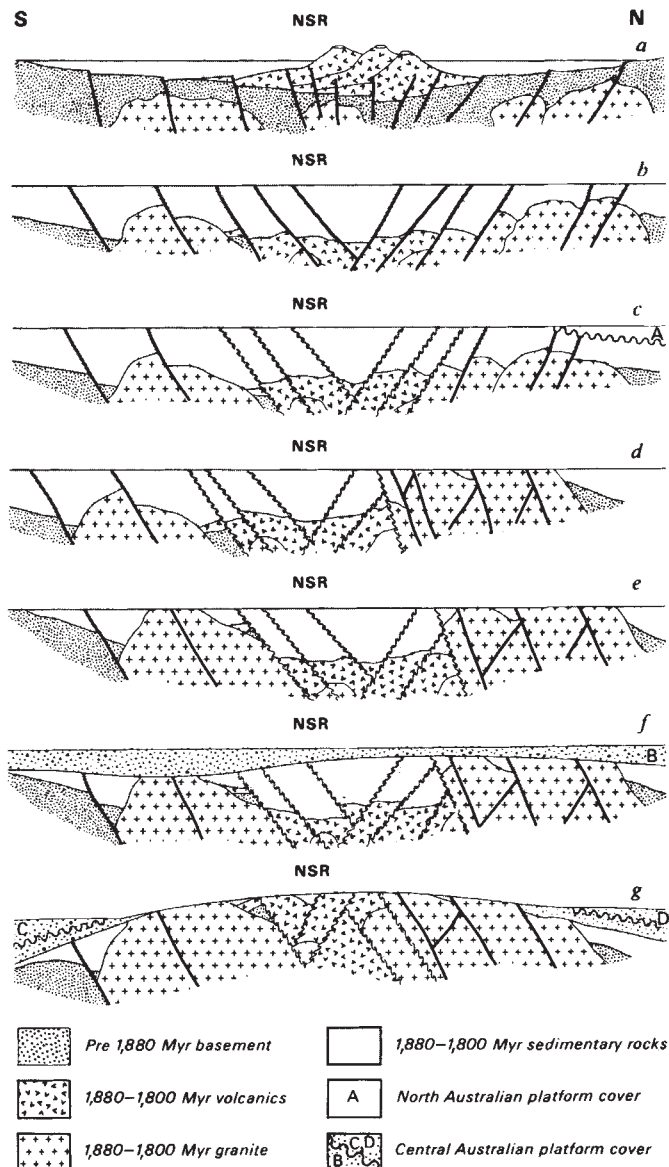


Fig. 2 The pressure-temperature paths interpreted from mineral assemblages found in the northern Strangways Range (W, western part of the northern Strangways Range; E, central-northern part of the Range, near the Edwards Creek Prospect). Stages recognized from mineral assemblages are: Gr, granulite; Bi, pervasive hydration characterized by biotite; Ky-Ged, localized hydration in shear zones characterized by kyanite and gedrite; Sil-Str, in which sillimanite and staurolite formed in cordierite which itself had formed from kyanite and gedrite. The temperature gap shown between the Gr and Bi stages is schematic, as the formation of biotite may have extended over a temperature interval. The time correlations (shown in *italics*) are based on the observation that the Cd-Ky-Ged and Sil-Str stages correlate with the uplift history of central Australia in the mid-Palaeozoic. This interpretation carries the rider that the assemblages of the kyanite-gedrite stage may have formed in all (or any) of the dated episodes of deformation adjacent to shear zones in the Arunta Block.

Table 1 Summary of stages in the metamorphic parageneses and their tectonic implication

| Stage                                                    | Essential assemblage/reaction                                                           | Other assemblages/reactions                                                                | Tectonic implication                                    |
|----------------------------------------------------------|-----------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------|---------------------------------------------------------|
| Granulite                                                | Opx-Kf                                                                                  | Opx-Kf-Sap $\pm$ Sil $\pm$ Cd<br>Scapolite-wollastonite-calcite                            | High heat flow, low $P_{H_2O}$ , high $P_{CO_2}$        |
| Biotite                                                  | $H_2O + Opx + Kf \rightarrow Bi + Q$                                                    | $H_2O + Opx + Kf + Sp \rightarrow Sap + Ph$<br>$H_2O + Opx + Kf + Sap \rightarrow Cd + Ph$ | Autoregression or influx of $H_2O$ ?                    |
| Isobaric cooling                                         | $Sap + Cd \rightarrow Opx + Sil$<br>$Cd \rightarrow Opx + Sil + Q$                      |                                                                                            | Isostatic equilibrium                                   |
| Kyanite-gedrite                                          | $Cd \rightarrow Ged + Ky + Q$                                                           | Scapolite-wollastonite $\rightarrow$<br>$Q$ -grossularite- $CO_2$                          | Normal geothermal gradient and<br>local influx of water |
| Cordierite-kyanite-gedrite<br>Sillimanite and staurolite | Cordierite enclosing kyanite<br>Sillimanite and staurolite<br>growing within cordierite |                                                                                            | Uplift<br>Isobaric cooling                              |
| Quartz-chlorite                                          | $Cd + Ged \rightarrow Ch + Q$                                                           |                                                                                            | Renewed hydration and deformation                       |

Bi, biotite; Cd, cordierite; Ch, chlorite; Ged, gedrite; Kf, potassium feldspar; Opx, orthopyroxene; Ph, phlogopite; Q, quartz; Sap, sapphirine; Sil, sillimanite; Sp, spinel.



**Fig. 3** Tectonic development of the Arunta Block as interpreted from the metamorphic history; shown by schematic cross-sections (not to scale) drawn across the northern Strangways Range (NSR) from the Georgina Basin to the Amadeus Basin. *a*, Formation of protoliths (1,880–1,800 Myr). Rift-like structure initiated by intrusion of hot mantle-differentiates into the base of the crust<sup>4,7</sup>, and probable attenuation of the lower crust. Fractionation and partial melting of the mantle-differentiates produced acid volcanism (central part of the rift) and penecontemporaneous granites which began to move upwards (outer margins of the rift). Sediments derived partly from the volcanics and partly from external sources infilled the rift. *b*, Stabilization of the rift (~1,800 Myr)<sup>2,3</sup>. As attenuation of the lower crust continued, the volcanics were downfaulted and the rift filled by sediments; with waning of the extensional tectonic regime, folding occurred. Heat and (possible) CO<sub>2</sub> dissipated during cooling of the mantle-derived intrusions metamorphosed the folded rocks. *c*, Initiation of shear zones (~1,700 Myr)<sup>2</sup>. A normal geothermal gradient was established once the cooling of the heat source was complete. Retrogressive metamorphism and hydration along shear zones (probably reactivated faults of the rifting stage) marked the change from regional to localized hydration<sup>1</sup>. North Australian Platform Cover (A) was deposited on the northwestern margin of the Arunta Block at ~1,700 Myr (ref. 23), but has not been recognized in the region shown in the section line. *d–g*, Uplift and unroofing. Uplift of the northern margin of the Arunta Block (*d*) is probably recorded by K–Ar ages ~1,450 Myr (ref. 24). Uplift of the southern Arunta Block (*e*) followed deformation ~1,000 Myr (refs 22, 25) and predated (*f*) deposition of Central Australian Platform Cover from after 900 Myr (ref. 26) to late Ordovician<sup>20</sup>. Major deformation in the mid-Palaeozoic caused (*g*) the formation of troughs (C and D) to the north and south, and nappes (not shown) which moved south over the southern trough<sup>20</sup>. *d–g* Have been drawn as if the major uplift of the central zone occurred in the mid-Palaeozoic, based on the correlation between metamorphic evolution and mid-Palaeozoic uplift (Fig. 2). Present data, however, do not conclusively exclude the possibility that the central zone Hwas uplifted either with the northern zone, ~1,450 Myr or with the southern zone, ~1,000 Myr.

is commonly followed by formation of oceanic crust, which is not recognized in the Arunta Block. Nevertheless, basement beneath the volcanic complexes must have been removed so that they were buried to 25–30 km in isostatic equilibrium. The large volume of mantle-derived material that differentiated to form the volcanics would have also provided a source for the high heat flow necessary to produce the high temperatures of the granulite stage. The mid-Proterozoic tectonic regime in which the Arunta rocks formed and were metamorphosed therefore appears to have been broadly hotspot<sup>9</sup> in nature, but different from modern rift-systems causing continental breakup<sup>16</sup>.

Once the heat source had cooled, the tectonic activity was spasmodic and confined to the major shear zones, which continued to develop until the rocks were returned to near surface levels.

Even though areas north and south of the Strangways Range were uplifted before the end of the Proterozoic (Fig. 3*d, e*), the rocks in the northern Strangways Range may have remained deeply buried until the mid-Palaeozoic when there was major deformation in central Australia<sup>20</sup>. The unroofing of the rocks of the northern Strangways Range as deduced from mineral parageneses correlates remarkably well with the tectonic history of the region as interpreted from sedimentation in the Amadeus Basin (from ref. 20):

(1) By the end of the Ordovician the Arunta Block was overlain by 2–4 km of platform sediments.

(2) In the Silurian or early Devonian (Rodingan movement) the Arunta Block was uplifted, but not sufficiently to expose the basement. This uplift of <4 km is very similar to that in which the cordierite–kyanite–gedrite stage assemblages were formed.

(3) The Rodingan movement was followed by peneplanation. This corresponds to the episode of isobaric cooling in which sillimanite and staurolite formed in cordierite.

(4) Finally, there was massive uplift of the central Arunta Block with overthrusting and movement of nappes southwards over the Amadeus Basin. This is correlated with the formation of quartz and chlorite in intensely deformed parts of the schist zones, and final unroofing.

Such a correlation implies total uplift from the Ordovician to the early Carboniferous to have been ~25 km (that is, equivalent to 8 kbar pressure); a similar uplift has also been estimated from a model based on large-scale crustal buckling<sup>21</sup>.

Such a correlation also carries the implication that the development of the kyanite–gedrite assemblages in the schist zones extended from ~1,700 Myr, the earliest recorded age for amphibolite retrogression<sup>2</sup>, until the Rodingan movement. Published ages ~1,400 Myr from Johannsens Phlogopite Mine (southern Strangways Range)<sup>3</sup>, where gedrite-stage assemblages are abundantly developed along fracture zones, and of about 1,000 Myr from the Harry Creek Deformed Zones<sup>22</sup>, suggest that there was more than one age of major movement and hydration concentrated along the shear zones.

This account of the metamorphic parageneses in the northern Strangways Range and its implications for the tectonic evolution of the Arunta Block is based on research for a thesis at the University of New South Wales and on regional geological work for the Bureau of Mineral Resources, Geology and Geophysics and is published with permission of the bureau's director.



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## The Mesozoic marginal basin of central Peru

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The west Peruvian Trough<sup>1,2</sup> extends some 1,300 km from north of Trujillo to about 150 km short of Arequipa (Fig. 1). It received upwards of 9,000 m of material during the Mesozoic and within the Cretaceous rocks there is a well defined west-to-east facies change whereby thick, volcanoclastic turbidites and pillow lavas (the 'eugeosyncline' of Cobbing<sup>3</sup>) thin eastward to be replaced by a mixed clastic shelf sequence (the 'miogeosyncline'). We report here chemical analyses of the volcanic rocks and data on the association of cherts, volcanoclastic turbidites, dyke and sill complexes and large gabbro masses as well as geophysical evidence which suggests that the 'eugeosyncline' is an extensional marginal basin similar to that seen in rocks of the same age in Southern Chile<sup>4</sup>. The Coastal Batholith is thought to have been emplaced within this marginal basin during the Upper Cretaceous<sup>5</sup>, and the evolution of this 'ensialic' new crust therefore has important implications for the growth of the continental margin of South America, as well as for batholith genesis.

The two major basins of deposition in the western part of the West Peruvian Trough are those of Huarmey (400 km long) and Cañete (300 km long, Fig. 1), which are separated although interconnected<sup>3</sup>. Here only the Huarmey basin is considered. Near Lima it is made up of a lava/volcanoclastic turbidite sequence interrupted by a coarse-grained clastic wedge<sup>6</sup>.

The upper part of the sequence (Casma Formation) is made up of pillow lavas (dominant), hyaloclastites, turbiditic tuffs, limestones, cherts, siliceous ashes and foraminiferal muds<sup>3,7</sup>. Towards the top there is a change to subaerial volcanism.

The volcanoclastics thicken to the west and limestones, shales, sandstones and subaerial pyroclastics come in towards the eastern side of the basin. Clasts diminish to the east and turbidity currents are axial or from the west. Relatively acid volcanism towards the platform or continental side is synchronous with tholeiitic/calc-alkali volcanism in the basin. Throughout the sequence there are contemporaneous dykes and sills of similar composition to the lavas. The dykes are vertical or dip steeply to the west, are up to 8 m or more in width, often occurring in swarms, with an Andean trend, along extended fracture systems, and form a significant part of the total outcrop.

A volumetrically important component of the basin are gabbros, which occur as dykes, plugs, plutons and flat-topped arcuate-type bodies. They postdate the deformation in the basal rocks and predate the Coastal Batholith<sup>8</sup>. Studies of the metamorphism of the volcanic rocks have indicated that it was produced by high geothermal gradients.

Beneath the Casma Formation at the southern end of the basin, below the coarse-grained clastic wedge, is the Puente Piedra Formation (Berriasian): 2,000 m of basic lavas, pillow lavas, acid tuffs and andesites with intercalations of limestone and shale. These rocks constitute the oldest rocks seen in the Huarmey basin. Of the lavas analysed from the Huarmey basin, 55% are basalts, 31% basaltic andesites, 7% andesites and 7% are dacites<sup>10</sup> (Fig. 2). They contain phenocrysts of plagioclase, pyroxene, and rarely, magnetite and hornblende; the latter often after pyroxene, set in a matrix of plagioclase, pyroxene, rare hornblende, quartz, magnetite and in some cases glass. Plagioclase phenocrysts dominate and may exceed 2 mm in length. Olivine is rare, although skeletal opaques with iddingsite may indicate its previous existence in some rocks. The norms confirm the modal evidence in that the rocks are mainly normative two pyroxene-feldspar mixes, mostly plus quartz, although a few of the more primitive basic rocks are olivine normative. They lie in the plagioclase tholeiite field of Shido<sup>11</sup>. The rocks from the Casma Formation have low K or calc-alkali characteristics, while those from the Puente Piedra Formation are more variable, but are distinguished in most cases by their high K contents (Fig. 2).

Most rocks lie in the high  $Al_2O_3$  field of Kuno<sup>12</sup>, except for the alkaline rocks, and in the tholeiite or calc-alkaline field on an AFM [(Na<sub>2</sub>O + K<sub>2</sub>O)–FeO–MgO] diagram<sup>10</sup>. The low Zr, Ti and P<sub>2</sub>O<sub>5</sub> (<49 p.p.m., <0.7% and <0.09%, respectively) and moderately enhanced levels of large ion lithophile (LIL) elements (K<sub>2</sub>O <0.25%, Rb <9 p.p.m., Ba <269), compared to mid-ocean ridge basalt (MORB), of the Casma basalts are characteristic of island arc tholeiites (IAT). However, the high Cr and Ni and low Fe/Mg in the most primitive basalts (up to 466 p.p.m., 166 p.p.m. and 0.71, respectively) are different to IAT (Table 1). The rare earth elements (REEs) are flat with

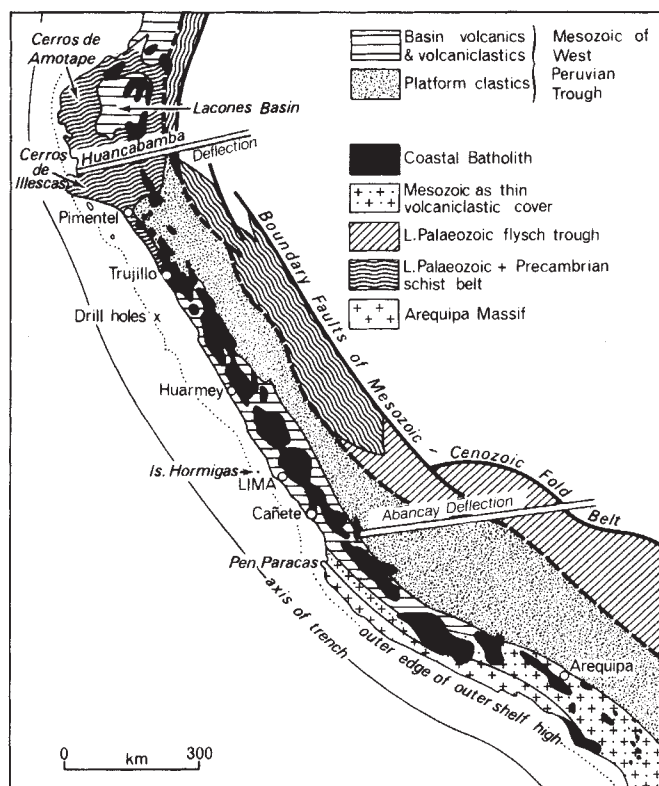


Fig. 1 Simplified geological map of Peru showing the outcrop of the Mesozoic marginal basin volcanics and volcanoclastics into which the Coastal Batholith is intruded, the Precambrian Arequipa massif and the outer edge of the outer shelf high<sup>1</sup>.

values 6–12 times chondrite (Fig. 3). These basalts alternate with basalts having lower Cr and Ni contents as well as enhanced levels of LIL to high field strength (HFS) elements. These latter rocks show slight REE enrichment ( $La_N/Yb_N \approx 2.0$ ) and near 'flat' curve for heavy REEs (HREEs) with values near 10 times chondrite. Europium anomalies in all rocks are characteristically absent (Fig. 3).

Chemically the Puente Piedra rocks are a diverse suite of basalts and andesites quite different to the Casma rocks. Most are high K rocks ( $K_2O > 1.0\%$ ) although some are calc-alkali (Fig. 2). They have strongly enhanced levels of LIL to HFS elements and are strongly depleted in Ni and Cr, and show moderate enrichment in light REEs (LREEs) (Fig. 3). The high Ti, and to a lesser extent Y, as well as enrichment in Sr, K, Rb, Th, Ta, Nb, Cr, P, Zr, Hf and Sm, compared to MORB (Table 1), clearly indicate their alkali nature and together with the enrichment in low ionic potential elements suggest both island arc and within-plate tholeiitic components. REEs for five basalts are shown in Fig. 3, from which it can be seen that the Puente Piedra basalts are slightly LREE-enriched ( $Ce_N/Yb_N \approx 2.0$ ) with flat HREE, about 10 times chondrite.

The transitional or 'anomalous' character<sup>13</sup> of the Puente Piedra rocks is similar to that seen in magmas from within-plate environments, for example, Bogoslov Island (Table 1), or adjacent to continental areas during the initial stages of back-arc rifting or spreading (Penguin Island in the Bransfield Strait<sup>14</sup>; Sarmiento, S. Chile<sup>15</sup>). In contrast, the Casma rock chemistry

is very similar to some 'ensialic' back-arc basins in its transitional 'arc'-like signature<sup>16</sup>, produced when spreading was initiated on splitting of a calc-alkaline volcanic arc. In these respects the rocks are similar to basalts from Sarmiento, Bransfield Strait and the inner part of the Gulf of California and Scotia Sea<sup>17</sup> (Table 1) and appear to have been generated in mantle source regions enhanced in LIL elements and on occasion LREE, but not HFS elements. These features presumably relate to enrichment from the downgoing slab at an earlier period.

Previously, it was thought that the Huarmey and Cañete basins rested on old thick, block-faulted continental crust<sup>3</sup>. Certainly in southern Peru, part of the Cañete basin rests unconformably on the Arequipa massif and its Palaeozoic cover<sup>18,19</sup> (Fig. 1). In north-west Peru metamorphic schists and gneisses of the Cerros de Amotape and Illescas are thought to be equivalent and are linked by a structural ridge on the submarine shelf<sup>20</sup>, the lithologies of which, from submarine drill cores and outcrops on offshore islands, for example, Pimentel and Isla Hormigas (Fig. 1), are similar to those of the Amotape Mountains to the north.

During the lower Cretaceous this ridge formed a positive area as indicated by the easterly clast size diminution and the presence of granite pebbles within the basinal sediments<sup>3,21</sup>. During the Valanginian, plant-bearing sandstones with conglomeratic horizons and easterly-directed current bedding foresets<sup>2</sup> indicate emergence of this block, which is confirmed by the isolated fauna of the basin compared to that of the western ocean<sup>6</sup>.

The relation of the offshore ridge to the basins has been revealed by the recent gravity anomaly modelling of the continental margin at 9° S, 12° S, Pisco and Mollendo<sup>22,23</sup> which is shown schematically in Fig. 4. In the northern traverse at 9° S the outer continental shelf high is a complex structure including

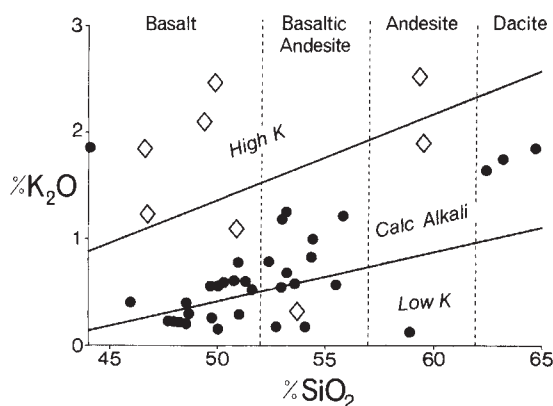


Fig. 2  $K_2O/SiO_2$  plot of the Casma (filled circles) and Puente Piedra (diamond) rocks, indicating the basic character of both formations and their low-K and high-K character respectively.

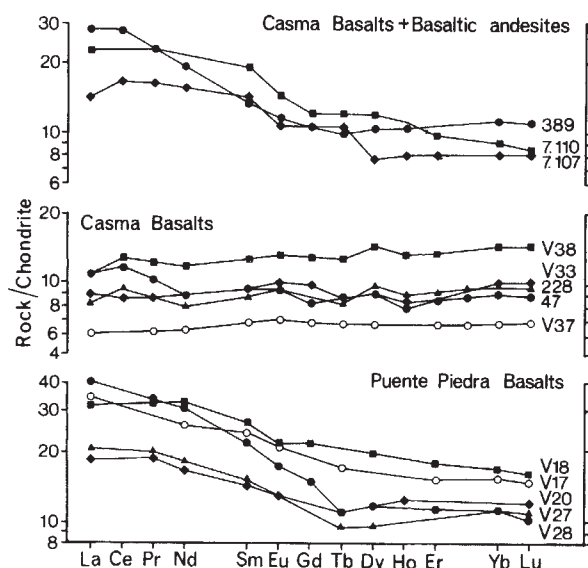


Fig. 3 REE plots of Casma basalts and basaltic andesites compared to Puente Piedra basalts.

Table 1 Representative Peru marginal basin rocks compared with other 'back-arc' rocks

|                                | 1      | 2     | 3      | 4     | 5     | 6      | 7     |
|--------------------------------|--------|-------|--------|-------|-------|--------|-------|
| SiO <sub>2</sub>               | 53.40  | 49.63 | 53.58  | 48.19 | 49.31 | 48.90  | 49.86 |
| TiO <sub>2</sub>               | 0.92   | 0.85  | 0.60   | 0.48  | 0.84  | 1.29   | 1.71  |
| Al <sub>2</sub> O <sub>3</sub> | 14.21  | 14.26 | 14.65  | 14.67 | 17.93 | 18.00  | 18.41 |
| Fe <sub>2</sub> O <sub>3</sub> | 8.79   | 9.92  | 9.52   | 1.04  | 4.31  | 5.45   | 6.34  |
| FeO                            | ND     | ND    | ND     | 7.44  | 4.57  | 4.73   | 2.33  |
| MnO                            | 0.23   | 0.18  | 0.17   | 0.13  | 0.16  | 0.16   | 0.18  |
| MgO                            | 6.49   | 9.30  | 8.58   | 10.33 | 5.79  | 5.30   | 4.18  |
| CaO                            | 9.39   | 10.40 | 10.67  | 12.10 | 8.14  | 11.72  | 6.89  |
| Na <sub>2</sub> O              | 4.72   | 1.33  | 1.91   | 1.45  | 3.25  | 2.91   | 4.20  |
| K <sub>2</sub> O               | 0.56   | 0.26  | 0.19   | 0.22  | 2.08  | 1.80   | 2.46  |
| H <sub>2</sub> O               |        |       |        |       | 1.95  |        | 2.19  |
| CO <sub>2</sub>                |        |       |        |       | 0.10  |        | 0.22  |
| P <sub>2</sub> O <sub>5</sub>  | 0.12   | 0.21  | 0.08   | 0.05  | 0.11  | 0.34   | 0.44  |
| IL                             | 1.51   | 3.17  |        |       |       | 0.27   |       |
| Total (%)                      | 100.84 | 99.51 | 99.95  | 96.10 | 98.54 | 100.06 | 99.41 |
| Fe*/Mg (p.p.m.)                | 1.22   | 1.96  | 1.00   | 0.81  | 1.46  | 1.82   | 1.92  |
| Ba                             | 93     | 84    | 49     | 269   | 432   | 888    | 1259  |
| Ce                             | 17     | 13    |        | 6     | 24    | 31     | 28    |
| Co                             |        |       |        | 43    | 35    | 32     | 32    |
| Cr                             | 170    | 433   | 321    | 373   | 47    | 39     | 76    |
| La                             | 7      | 6     |        | 1     | 6     | 7      | 8     |
| Ni                             | 34     | 123   | 62     | 96    | 5     | 7      | 18    |
| Pb                             | 3      | 2     | 3      | 8     | 7     | 7      | 17    |
| Rb                             | 8      | 10    | 4      | 5     | 51    | 51     | 44    |
| Sr                             | 177    | 148   | 118    | 222   | 514   | 841    | 473   |
| Th                             | 3      | 3     | 2      |       | 1     | 6      | 4     |
| V                              |        |       |        | 235   | 293   | 344    | 191   |
| Y                              | 23     | 20    | 14     | 14    | 26    | 26     | 36    |
| Zn                             | 30     | 69    | 64     | 79    | 108   | 69     | 134   |
| Zr                             | 88     | 113   | 37     | 26    | 71    | 126    | 193   |
| Nb                             | 2      | 2     | 1      |       | 4     | 3      | 14    |
| K/Rb                           | 249    | 216   | 394    | 365   | 344   | 292    | 462   |
| Rb/Sr                          | 0.05   | 0.07  | 0.03   | 0.02  | 0.97  | 0.06   | 0.09  |
| (Ce/Yb) <sub>N</sub>           | 2.1    | 1.9   | (0.94) | 0.90  | 1.5   | 4      | 2.3   |

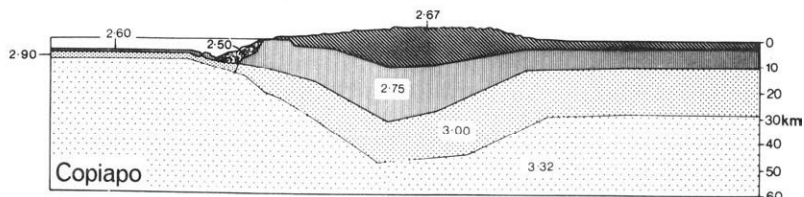
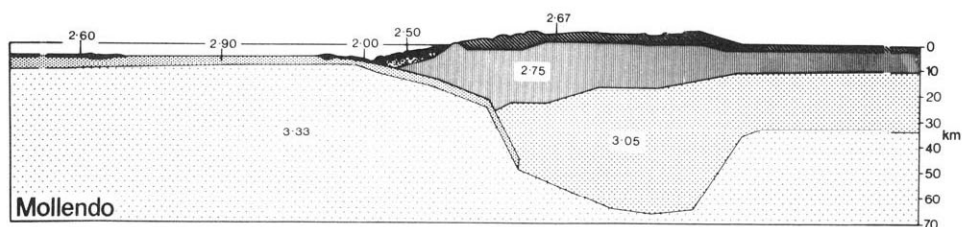
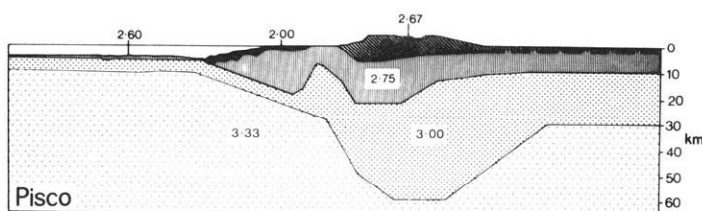
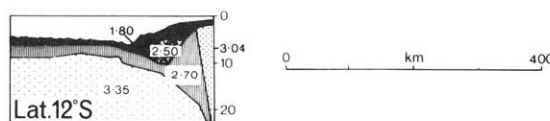
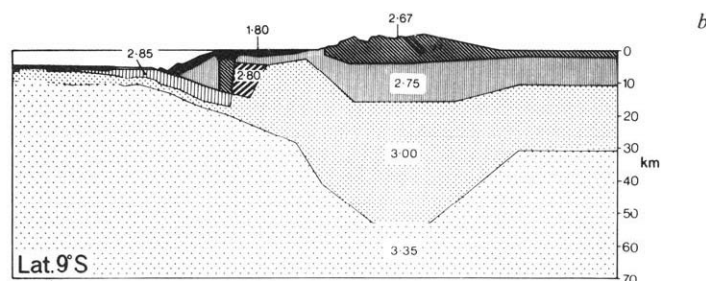
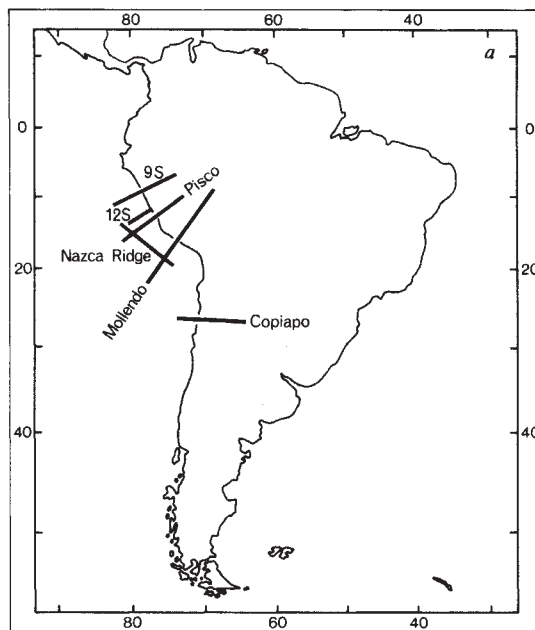
- (1) Lava (PL2), Sarmiento ophiolite complex<sup>15</sup>.
  - (2) Dike (PA230), Sarmiento ophiolite complex<sup>15</sup>.
  - (3) Basalt from dredge 24, South Sandwich spreading centre (D24.11)<sup>26</sup>.
  - (4) Basalt, Casma Formation, Quebrada Quilca, near Lima.
  - (5) Basalt, Puente Piedra Formation, Mala, 50 km south of Lima.
  - (6) Basalt, Bogoslov volcanic centre, Aleutian Islands<sup>33</sup>.
  - (7) Basalt, Puente Piedra formation, Punta Lobos, south of Lima.
- IL, loss on ignition; Fe\*/Mg, Fe as Fe<sup>2+</sup>. ND, not determined.



three basement wedges separated by faults, the most landward, with the highest density, being Palaeozoic or perhaps Precambrian. The Tertiary Salaverry basin lies to the east of this high and is floored by a basement of density  $2.75 \text{ g cm}^{-3}$  shelving to the east, which Jones<sup>22</sup> thought was made up of pillow lavas, cherts and pyroclastics of Mesozoic age continuous with the exposure on land, that is, Huarmey basin. Importantly, underneath the latter is a large arch-like structure of  $3.0 \text{ g cm}^{-3}$  rock which Jones considers may indicate crustal rupture and imbricate upthrust of oceanic crust or intrusion at depth<sup>22,23</sup>.

Further south at  $12^\circ \text{S}$  and Pisco an upbowing of dense material is also present (Fig. 4) which Couch *et al.*<sup>23</sup> consider could be either upper mantle material resulting from fracturing or thrusting during subduction, or lower crust (gabbroic or mafic) emplaced at a high level.

This structure is absent in the Mollendo section, near Arequipa (Fig. 4); rather there is a flat  $2.75 \text{ g cm}^{-3}$  wedge thickening towards the west, which is presumably the cratonic Arequipa massif. The section further south in Chile<sup>23</sup> shows no indication of crustal thinning or rupture, although here the  $2.75 \text{ g cm}^{-3}$  element thins to the west. Thus, north of the Nazca Ridge there is a relatively dense wedge of rocks in the continental crust which widens and shoals to the north, centred near the coast at  $14^\circ \text{S}$  and mainly offshore at  $9^\circ \text{S}$ . To the south, at Mollendo, the structure is lacking.



**Fig. 4** *a*, Location map of the crustal and subcrustal sections shown in *b*. *b*, Crustal and subcrustal cross-sections of the South American continental margin from gravity modelling, simplified from refs 23, 24, 28. Vertical exaggeration is  $\sim 5:1$ .

A consequence of this splitting of the upper crust is the isolation of a thin slice of continental crust, now forming the outer shelf high, which is connected to the Arequipa massif to the south of Pisco and splays out to the north. Clearly high-density basic material has pierced the upper crust to progressively higher levels northwards of the Abancay deflection. This coincides with increasing subsidence in the basin above this structure, intense submarine volcanicity and metamorphism related to high geothermal gradients<sup>9</sup>.

The systematic change northwards is related to the structural segmentation of the Andes which results in considerable differences in crustal configuration between the segments. Thus the Abancay (14° S) and the Huancabamba (7°30' S) deflections (Fig. 1) are transverse lineaments that isolate a central Peruvian segment. Such lineaments represent late Palaeozoic shear zones<sup>24</sup> that were reactivated during the Mesozoic as axes of uplift causing, for example, the deposits of the Huarmey Basin to thin northwards against the Huancabamba axis, and those of the Cañete Basin to change facies southwards approaching the Abancay deflection. More fundamentally, the latter may represent the line of a change in the pre-Mesozoic crust because there are certainly significant differences in the present crustal structure across the Abancay deflection<sup>22</sup> (Fig. 4).

It seems that the crustal configuration of the central Peruvian segment in the Mesozoic was different from that of its southern and northern counterparts. Here the crust was presumably thinning and/or splitting so that basinal development occurred within two arms of old crust to the north and south of the Huancabamba and Abancay deflections respectively (Fig. 1).

In many ways the Peruvian basin closely resembles the late Jurassic to early Cretaceous 'back arc' extension in the Southern Andes<sup>4</sup> both in time and rock types. New crust in both cases consists mainly of basaltic pillow lavas, dykes, sills and gabbros. Ultramafic rocks are absent and volcanoclastic turbidites typical. The absence of ultramafic rocks may relate to the erosion level, that is, the ultramafics may occur at depth, as part of the high-density 3.0 g cm<sup>-3</sup> material, which itself may be mainly lower crust flowing in from the east, or upthrust, underplated material<sup>10</sup>. The basin is not therefore 'ophiolitic' in the strict sense, and closure did not occur due to protection afforded by the pre-Mesozoic blocks to the north and south of the two major deflections.

It is not possible to be certain about the cause of splitting/subsidence. The overall long-standing tensional environment during basin formation and later during the intrusion of the batholith is well documented<sup>3,7</sup>. Whether this extensional regime relates to subduction is not clear, although the initial opening which occurred about the time the Puente Piedra volcanics were formed probably was, as the within plate/arc chemistry is very evident.

Alternatively, the continent may formerly have had a much greater westerly extent<sup>25,26</sup>. Certainly the argument for an 'erosion' of the continental margin is clear<sup>27,28</sup> although the presently exposed continental edge has been *in situ* for a long time. Removal of this western part of the continent is possible by northerly movement along a major strike-slip fault<sup>29,30</sup>, which may well follow subduction along the same plate margin. In this context it is interesting to note that the thickness of the Arequipa massif increases towards the west and is thickest at the continental margin (Fig. 4). This feature, together with the long sliver-like mass of Palaeozoic or Precambrian rocks forming the shelf high in the northern segment is compatible with strike-slip movement and 'rip-off' features (compare with Andaman Sea).

Whatever the exact mechanism, partially 'encratic' marginal basin development is important in the growth of the western margin of South America. Thus, from the end of the Palaeozoic<sup>4,31,32</sup>, lithospheric extension and major crustal fragmentation has been a dominant process along the margin of South America and rifting undoubtedly appears to have been associated with subduction, plus probably strike-slip movement during the Mesozoic, reverting to subduction at the present day.

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## Silver distributions and fluxes in north-east Pacific waters

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**In recent years, significant knowledge has been gained about the oceanic distributions of several trace elements<sup>1,2</sup>. However, relatively little is known about amounts of Ag in the ocean, and how this element cycles through it. We report here that Ag levels are relatively low near the surface (~1 pmol kg<sup>-1</sup>), and that concentrations more or less steadily increase with depth; for example, 23 pmol kg<sup>-1</sup> at 2,440 m, the deepest sample we collected. In general, Ag depth profiles are similar to those observed for Cu (Fig. 1). The near-surface cycles of Ag appears to be involved with particulate organic matter uptake-sinking-regeneration processes.**

The Ag concentrations that we measured in profiles off central California (36.5° N, 123° W; May 1981), and off central Mexico (18° N, 108° W; October 1981) agree well with five Pacific Ocean values recently reported by Murozumi<sup>3</sup>. However, Murozumi's methods were completely different from ours; his samples were collected with the Patterson-Schaulé ultraclean common Pb sampler; Ag was preconcentrated using dithizone/chloroform and analyses were via isotope dilution mass spectrometry. Our water collections were made using 30-l Go-Flo samplers; preconcentration was by ammonium 1-pyrrolidine dithiocarbonate-diethylammonium diethyldithiocarbonate (APDC-DDDC)/chloroform organic extraction; and analyses were done using atomic absorption spectrophotometry (see ref. 4 for complete details). Our Ag blanks were 0.08 ± 0.05 total pmol (*n* = 10) compared with 0.16 total pmol found in our lowest sample. Although precision was not formally estimated, duplicated analyses were usually within 20% of each other for each depth sampled (Table 1). Yields of



$98 \pm 1\%$  were obtained when the organic extraction preconcentration technique was checked using  $^{110m}\text{Ag}$ .

In addition to the general (near-surface depth intervals = 100 m) vertical profiles shown in Fig. 1, we also measured Ag amounts in a detailed (near-surface depth intervals = 10 m) profile off Mexico (Fig. 2). Much of the variability in Fig. 2 probably results from analytical imprecision at these very low levels; nevertheless, certain features are obvious. Silver concentrations are of the order of  $1.1\text{--}1.5\text{ pmol kg}^{-1}$  in the upper 40 m. Values then drop sharply until a minimum of  $0.4\text{ pmol kg}^{-1}$  is observed

at 60 m. Amounts of Ag increase to  $\sim 2\text{ pmol kg}^{-1}$  at 100 m, and generally decrease until a secondary minimum ( $0.8\text{ pmol kg}^{-1}$ ) is reached at 300 m. Concentrations then steadily increase with depth for the remainder of the water column sampled; major features of the Ag profile appear to be the same as those for Cu (Figs 1, 2).

Total suspended particulate Ag concentrations measured in the Mexico samples (Table 1) following  $0.4\text{ }\mu\text{m}$  Nucleopore filtration usually mirrored dissolved distributions. Silver in the particulate form generally represented 10% or less of the total present, with the exception of the dissolved minima/particulate maxima at 60, 150 and 245 m, where percentages of particulate Ag were of the order of 30% (Table 1).

The Ag data shown in Fig. 2 arise from a complex interaction of horizontal and vertical advection-diffusion processes, and particulate uptake-sinking-regeneration processes. Based on Wyrki's<sup>5</sup> descriptions of the area, three distinct water masses are found in the upper water column: subtropical surface water from 0 to 70 m (salinity,  $S = 34.31$ ); subtropical subsurface water from 120 to 270 m ( $S = 34.76$ ); and Antarctic intermediate water beginning at 600 m ( $S = 34.56$ ). The mixed layer (0–25 m) is very thin in this region, and a steep thermocline/pycnocline occurs between 25 and 125 m (Fig. 2). Oxygen levels also fall rapidly from near saturation at 50 m to close to 0 at 120 m (Fig. 2). A distinct subsurface fluorescence maximum<sup>6</sup>, which corresponds to the chlorophyll maximum, has also been observed at 60 m. This feature coincides precisely with the dissolved Ag minimum (Fig. 2). In comparison with the phenomena described above, the waters between the subsurface salinity maximum and intermediate water are relatively featureless. Temperature-salinity plots between 300 and 600 m suggest near-conservative mixing processes between the two water types.

In addition to the water column hydrographic features described above, MLML particle traps<sup>7</sup> were set at several depths throughout the upper 1,950 m water column to determine the importance of particulate transport processes. Ag fluxes measured with these devices are shown in Fig. 3. Particulate Ag removal rates are highest near the surface ( $510\text{ pmol m}^{-2}\text{ day}^{-1}$  at 120 m), and then steadily decrease with depth to about  $60\text{ pmol m}^{-2}\text{ day}^{-1}$  at 1,950 m. This flux pattern is remarkably similar to that observed for organic carbon (Fig. 3), and molar ratios of Ag:C are consistent throughout the water column, averaging  $1.7 \pm 0.25 \times 10^{-7}$  for the eight depths sampled (Fig. 3).

The coincidence of the dissolved Ag minimum and fluorescence maximum (Fig. 2) together with the tightly coupled Ag

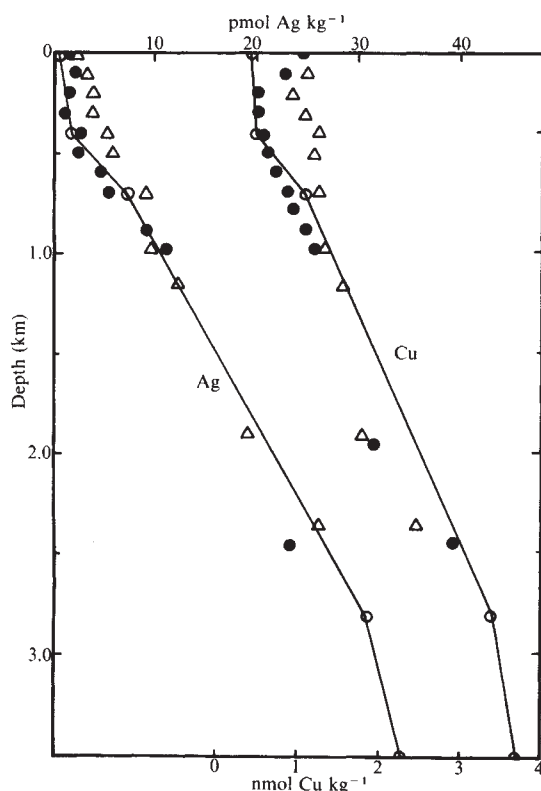


Fig. 1 Dissolved Ag concentrations versus depth measured at  $18^\circ\text{N}$ ,  $108^\circ\text{W}$  (●), and at  $36.5^\circ\text{N}$ ,  $123^\circ\text{W}$  (Δ) compared with those measured at  $20^\circ\text{S}$ ,  $160^\circ\text{W}$  (○) by Murozumi<sup>3</sup>. Copper levels for these locations are also shown for comparison.

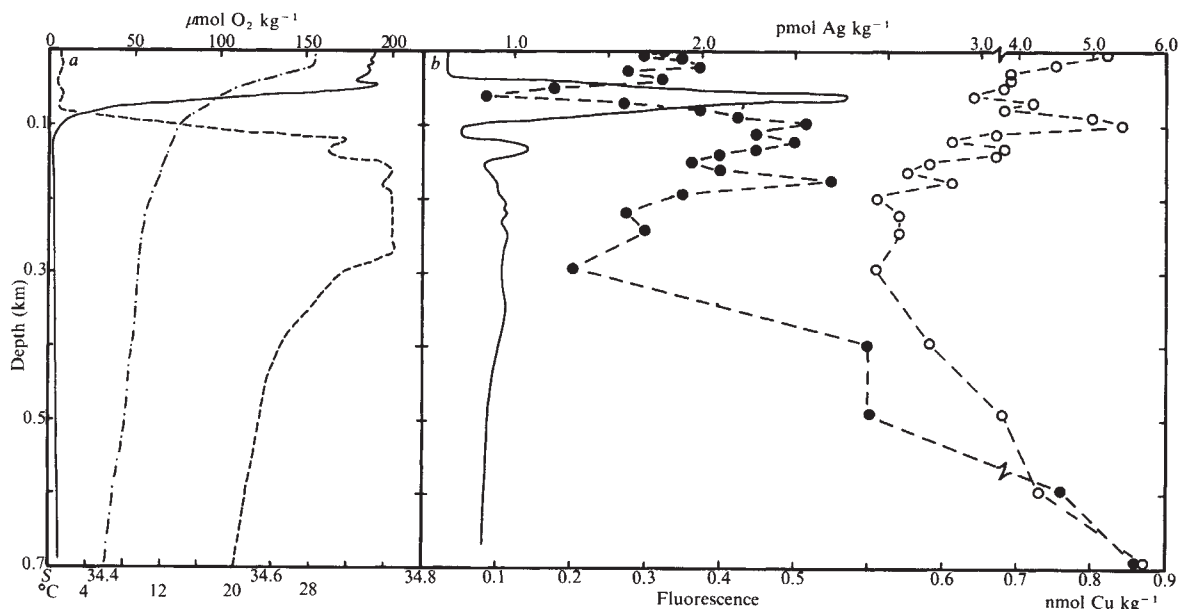
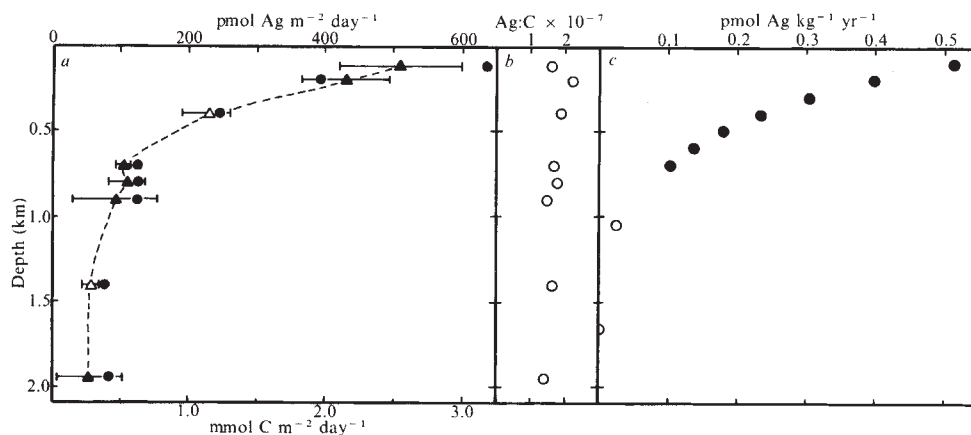


Fig. 2 a, Oxygen (—), salinity (---), and temperature (· · ·) data for this  $18^\circ\text{N}$ ,  $108^\circ\text{W}$  station. b, Near-surface dissolved Ag (●) and Cu (○) concentrations with fluorescence (for units see ref. 6) (—).



**Fig. 3** *a*, Silver and organic C (●) fluxes observed at 18° N, 108° W. Silver means  $\pm 1$  s.d. (▲) for three replicates, and means and ranges (Δ) for two replicates. *b*, Observed Ag:C ratios. *c*, Ag regeneration rates: 100–700 m regeneration rates (●) are from model (see text); others (○) represent finite difference rate-of-change estimates.

and organic C flux data imply Ag complexation with organic matter in the water column. Although we do not know if the Ag is incorporated within organic C matrices through active uptake processes or passively adsorbed on organic C surfaces, we do know that these Ag/C complexes are relatively strong, because trapped particulate Ag was released only after acid digestion in Teflon bombs<sup>8</sup>. Less than 6% of the total was released using a weak (25% acetic acid) leach technique<sup>9</sup>.

Examination of the flux data in Fig. 3 suggests an exponential decrease with depth, and when the logs of the 120–700 m Ag fluxes were plotted versus depth, the points fell along a straight line. Thus, these data can be expressed using the equation  $F = F_0 e^{-\alpha z}$  (ref. 10) where  $F$  is the flux at depth  $z$ ,  $F_0$  is the flux at zero model depth, and  $\alpha$  is the exponential decay constant. Using an  $F_0$  estimate of  $740 \text{ pmol m}^{-2} \text{ day}^{-1}$ , we calculated an  $\alpha \pm 1$  s.d. of  $2.8 \pm 0.2 \times 10^{-3} \text{ m}^{-1}$ .

**Table 1** Mean dissolved Ag concentrations (Ag  $x$ ) measured in two replicates (Ag 1, Ag 2) off Mexico (18° N, 108° W)

| Depth (m) | Ag 1 | Ag 2 (pmol kg <sup>-1</sup> ) | Ag $x$ | P Ag | %P Ag | Cu (nmol kg <sup>-1</sup> ) |
|-----------|------|-------------------------------|--------|------|-------|-----------------------------|
| 0         | 1.3  | 1.4                           | 1.4    | 0.09 | 6     | 1.07                        |
| 5         | 1.3  | 1.1                           | 1.2    | 0.14 | 10    | 0.82                        |
| 10        | 1.5  | 1.2                           | 1.4    | 0.11 | 7     | —                           |
| 20        | 1.4  | 1.6                           | 1.5    | 0.18 | 11    | 0.75                        |
| 30        | 1.2  | 1.0                           | 1.1    | 0.24 | 18    | 0.69                        |
| 40        | 1.1  | 1.4                           | 1.2    | 0.12 | 9     | 0.69                        |
| 50        | 0.8  | 0.5                           | 0.6    | 0.28 | 32    | 0.68                        |
| 60        | 0.3  | 0.5                           | 0.4    | 0.22 | 36    | 0.64                        |
| 70        | 1.0  | 1.3                           | 1.2    | 0.11 | 8     | 0.72                        |
| 80        | 1.4  | 1.6                           | 1.5    | 0.13 | 8     | 0.68                        |
| 90        | 1.5  | 1.9                           | 1.7    | 0.10 | 6     | 0.80                        |
| 100       | 1.4  | 2.7                           | 2.0    | 0.14 | 6     | 0.84                        |
| 110       | 1.5  | 2.0                           | 1.8    | 0.12 | 6     | 0.67                        |
| 120       | 1.7  | 2.3                           | 2.0    | 0.18 | 8     | 0.61                        |
| 130       | 1.3  | 2.2                           | 1.8    | 0.21 | 10    | 0.68                        |
| 140       | 1.7  | 1.5                           | 1.6    | 0.36 | 18    | 0.67                        |
| 150       | 1.6  | 1.3                           | 1.4    | 0.63 | 31    | 0.58                        |
| 160       | 1.8  | 1.4                           | 1.6    | 0.45 | 22    | 0.55                        |
| 175       | 2.1  | 2.3                           | 2.2    | 0.22 | 9     | 0.61                        |
| 195       | 1.3  | 1.7                           | 1.5    | 0.36 | 19    | 0.51                        |
| 220       | 1.3  | 0.8                           | 1.0    | 0.54 | 35    | 0.54                        |
| 245       | 0.8  | 1.5                           | 1.2    | 0.65 | 35    | 0.54                        |
| 295       | 0.6  | 1.0                           | 0.8    | 0.35 | 30    | 0.51                        |
| 395       | 2.6  | 2.1                           | 2.4    | 0.22 | 8     | 0.58                        |
| 490       | 2.8  | 2.0                           | 2.4    | 0.22 | 8     | 0.68                        |
| 590       | 5.0  | 4.2                           | 4.6    | 0.23 | 5     | 0.73                        |
| 690       | 5.8  | 5.5                           | 5.6    | 0.15 | 3     | 0.87                        |
| 785       | —    | —                             | —      | 0.07 | —     | 0.95                        |
| 880       | 9.5  | 9.1                           | 9.3    | 0.13 | 1     | 1.09                        |
| 980       | 11.1 | 10.4                          | 10.8   | 0.09 | 1     | 1.19                        |
| 2,440     | —    | 22.8                          | 22.8   | 0.07 | 0.3   | 2.89                        |

P Ag, total particulate Ag values; %P Ag, percentages of particulate Ag (particulate/particulate + dissolved  $\times 100$ ); Cu, dissolved Cu concentrations.

The depth derivative of the exponential flux model yields an estimate of regeneration rates ( $R = \alpha F_0 e^{-\alpha z}$ ) that varies from 0.5 at 100 m to 0.1  $\text{pmol kg}^{-1} \text{ yr}^{-1}$  at 700 m. Below model depth, finite difference rate-of-change estimates ( $\Delta \text{Ag} / \Delta t \approx \Delta F / \Delta z$ ) for the 700–1,400 and 1,400–1,950 m portions of the water column, give values of 0.025 and 0.0007  $\text{pmol kg}^{-1} \text{ yr}^{-1}$ , respectively.

The combined water mass, particle flux, and regeneration rate data suggest the following: Ag is not markedly depleted in the mixed layer, and we assume that its removal is balanced or exceeded by atmospheric input processes at rates of the order of 180  $\text{pmol m}^{-2} \text{ day}^{-1}$  (ref. 11). Concentrations fall between 40 and 70 m as Ag is removed by organic particulate uptake in the subsurface fluorescence maximum. These particles sink out, and the Ag is rapidly regenerated in concert with the destruction of the organic matter carriers, as evidenced by the decreasing Ag and C fluxes (Fig. 3), and decreasing oxygen levels (Fig. 2). This results in the dissolved Ag maximum occurring at 100 m. Amounts of dissolved Ag decrease in the 100–300 m depth interval as regeneration rates decrease.

Although regeneration rates continue to decrease with depth, mixing with the older Antarctic intermediate water begins at 300 m, and Ag levels begin their continuous increase with depth. Because Ag regeneration rates become negligible (0.0007  $\text{pmol kg}^{-1} \text{ yr}^{-1}$ ) between 1,400 and 1,950 m, the monotonically increasing concentrations with depth may indicate that Ag has a bottom source similar to that reported for Cu (ref. 12). Thus, advective–diffusive rather than *in situ* processes determine the distribution of Ag in our study area at depths  $> 300$  m.

It can also be argued that advective–diffusive processes are responsible for the dissolved Ag minimum occurring at 300 m. This minimum coincides precisely with the dissolved Mn maximum<sup>13</sup> which may well result from remobilization of Mn in oxygen–minimum continental-slope sediments in conjunction with horizontal mixing processes<sup>13,14</sup>. Because organic C fluxes must be higher in the coastal upwelling waters off Mexico than those we measured here (Fig. 3), it is logical to assume that sulphides are being formed in the same sediments from which Mn is being released. Both Ag and Cu are very insoluble in the presence of sulphides ( $K_{sp} \text{Ag}_2\text{S} = 1 \times 10^{-49}$ ;  $\text{CuS} = 8.5 \times 10^{-45}$ )<sup>15</sup>; thus it is conceivable that the removal of these elements in slope sediments, together with horizontal mixing, results in the minima we observed 370 km from the coast. This process could also partially explain why the vertical dissolved distribution of Ag is not similar to Cd and  $\text{PO}_4$  vertical distributions despite the similarities these three elements have with observed particulate organic C flux patterns (compare Fig. 3 with data in ref. 16). These complexities will be discussed in greater detail in a future paper (in preparation) that will compare distributions and fluxes of 10 trace elements in this study area.

Horizontal advective mixing processes may also be responsible for maintaining mass balance in the upper waters of our study area. If Ag is being removed from the surface at a rate of 510  $\text{pmol m}^{-2} \text{ day}^{-1}$ , and if atmospheric input fluxes are only of the order of 180  $\text{pmol m}^{-2} \text{ day}^{-1}$  (ref. 11), and if the



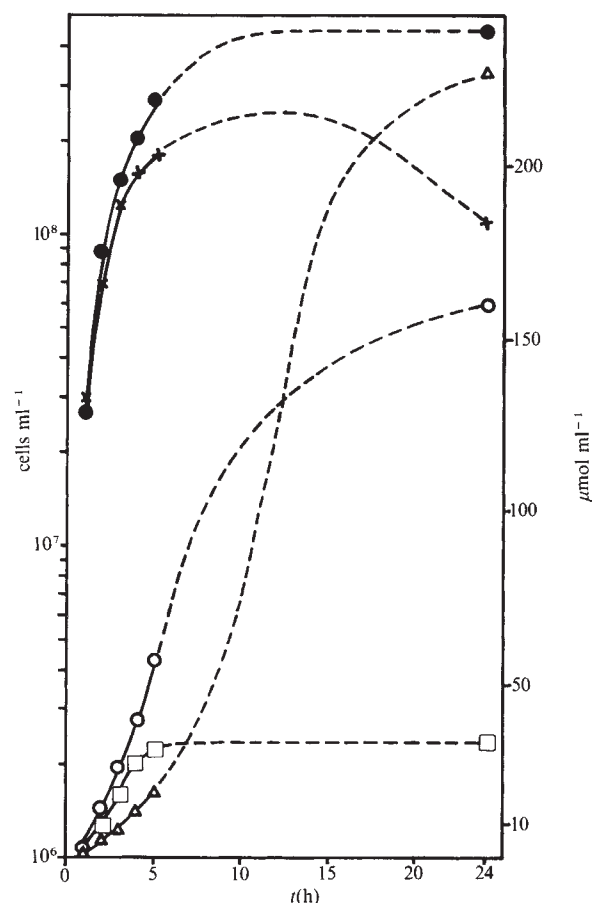
well-developed pycnocline (Fig. 2) prevents adequate vertical mixing, an additional input flux of  $330 \text{ pmol m}^{-2} \text{ day}^{-1}$  is needed to balance the output we measure. Although we assume that it is reasonable to invoke horizontal advective processes, we do not have the necessary horizontal distribution data to test this hypothesis.

The flux and water column data were also used to estimate Ag residence times. For example, total Ag in the upper water column (0–120 m) is of the order of  $160 \text{ pmol m}^{-2}$ . This value, divided by the 120 m Ag flux ( $190 \text{ nmol m}^{-2} \text{ yr}^{-1}$ ) results in a surface residence time of a little under 1 yr. Assuming that the average Ag concentration is  $\sim 22 \text{ pmol kg}^{-1}$  (Fig. 1) for a typical Pacific oceanic water column of 4,300 m, and that the particulate Ag flux at the bottom is about the same as the flux we measured at 1,950 m ( $18 \text{ nmol m}^{-2} \text{ yr}^{-1}$ ), a total water column Ag residence time of  $\sim 5,000 \text{ yr}$  is obtained. This time is the same as that estimated for Cu by Boyle *et al.*<sup>12</sup>, and again may point to the similarities between these Group Ib elements.

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**Fig. 1** Methane and  $\text{H}_2\text{S}$  formation during growth of *Methanococcus thermolithotrophicus*. ●, Growth without sulphur; ×, growth in the presence of sulphur; ○, methane formation without sulphur; □, methane formation in the presence of sulphur; △,  $\text{H}_2\text{S}$  formation in the presence of sulphur. The cells were grown with and without sulphur at  $65^\circ\text{C}$  as described in Table 1. Cell numbers were determined microscopically in a Thoma counting chamber.

## Reduction of molecular sulphur by methanogenic bacteria

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**Methanogenesis is the characteristic, energy-yielding metabolic pathway in methanogenic bacteria<sup>1</sup>. Here we report that in the presence of molecular sulphur, these bacteria form large amounts of  $\text{H}_2\text{S}$  by dissimilatory sulphur reduction, in addition to methane. Thus, methanogenic bacteria may be responsible for formation of much of the  $\text{H}_2\text{S}$  found in their habitats (marine sediments, sewage digestors and solfataric springs—where sulphur is present), and they may also be responsible for corrosion which has previously been thought to be due exclusively to sulphate-reducing bacteria. These extremely oxygen-sensitive organisms could also create their own anaerobic environment by  $\text{H}_2\text{S}$  formation. As both groups of anaerobic archaeobacteria—the anaerobic thermoacidophiles<sup>2</sup> and the methanogens—are able to reduce molecular sulphur, there may be a closer evolutionary relationship between the two groups than has been previously believed. Sulphur reduction seems to be a primitive means of energy conservation, and may have been the forerunner of energetically more efficient<sup>3</sup> methanogenesis.**

Some members of the eubacteria<sup>4–6</sup> and the anaerobic group of the branch of archaeobacteria containing the thermoacidophiles<sup>2,7,8</sup> are able to reduce molecular sulphur to  $\text{H}_2\text{S}$ . Within this group of archaeobacteria, the genera *Thermoproteus*, *Thermodiscus* and *Pyrodicticum* can exist simply on the basis of a hydrogen-sulphur autotrophy<sup>9</sup>, while the genera *Desulfurococcus*, *Thermofilum* and *Thermococcus* grow heterotrophically by sulphur respiration<sup>2</sup>. The second branch of the archaeobacteria contains the heterotrophic, aerobic extreme halophiles<sup>10</sup> as well as the three orders of strictly anaerobic methanogens<sup>1</sup>, which are known to gain energy by methane formation from  $\text{H}_2$  and  $\text{CO}_2$  or, less frequently, from the simple organic compounds formate, methanol, methylamines and acetate<sup>1</sup>. In nature, methanogens are found in thermal volcanic environments<sup>11,12</sup> and in decomposing organic matter in sediments, faeces and sewage digestors<sup>1</sup>. The volcanic biotope, possibly the oldest in the evolution of methanogens, consists of continental solfataric waterholes<sup>11,12</sup> and geothermally heated submarine sediments (ref. 13 and K.O.S., unpublished results), both well supplied with  $\text{H}_2$  and  $\text{CO}_2$  due to the volcanic exhalations. Methanogens thrive in such environments at neutral to slightly acidic pH ( $\sim 6.5$ ) in mixed cultures with thermophiles of the other branch of archaeobacteria such as *Thermoproteus neutrophilus* or *Thermodiscus maritimus*<sup>9</sup>, which reduce the sulphur that is present in large amounts (1–24%; ref. 14).

To investigate its possible influence on growth, we added molecular sulphur to exponentially growing cultures of methanogens, originally isolated from different biotopes (Table 1). Surprisingly, all methanogens of all three orders formed large quantities of  $\text{H}_2\text{S}$  in the presence of sulphur (Table 1),

**Table 1** Effect of sulphur on exponentially growing methanogenic bacteria

| Order                            | Species                                                 | Isolated from | A <sub>578</sub> * | Energy source      | Sulphur | Methane (μmol ml <sup>-1</sup> ) | H <sub>2</sub> S (μmol ml <sup>-1</sup> ) |
|----------------------------------|---------------------------------------------------------|---------------|--------------------|--------------------|---------|----------------------------------|-------------------------------------------|
| Methanobacteriales               | <i>Methanobacterium</i> sp. PL-10                       | vs            | 0.40               | H <sub>2</sub>     | —       | 226                              | —                                         |
|                                  |                                                         |               | 0.37               | H <sub>2</sub>     | +       | 36                               | 152                                       |
|                                  | <i>Methanobacterium</i> sp. R1/1                        | vc            | 0.38               | H <sub>2</sub>     | —       | 209                              | —                                         |
|                                  |                                                         |               | 0.41               | H <sub>2</sub>     | +       | 32                               | 114                                       |
|                                  | <i>Methanothermus fervidus</i> <sup>12</sup>            | vc            | 0.44               | H <sub>2</sub>     | —       | 209                              | —                                         |
|                                  |                                                         |               | 0.44               | H <sub>2</sub>     | +       | 1                                | 96                                        |
|                                  | <i>Methanobrevibacter smithii</i> <sup>1</sup>          | c             | 0.21               | H <sub>2</sub>     | —       | 81                               | —                                         |
|                                  |                                                         |               | 0.21               | H <sub>2</sub>     | +       | 6                                | 3                                         |
|                                  | <i>Methanobrevibacter</i> sp. N1                        | c             | 0.28               | H <sub>2</sub>     | —       | 109                              | —                                         |
|                                  |                                                         |               | 0.37               | H <sub>2</sub>     | +       | 33                               | 36                                        |
| Methanococcales                  | <i>Methanococcus voltae</i> <sup>1</sup>                | s             | 0.51               | H <sub>2</sub>     | —       | 92                               | —                                         |
|                                  |                                                         |               | 0.34               | H <sub>2</sub>     | +       | 47                               | 15                                        |
|                                  | <i>Methanococcus thermolithotrophicus</i> <sup>13</sup> | vs            | 0.78               | H <sub>2</sub>     | —       | 239                              | —                                         |
|                                  |                                                         |               | 0.79               | H <sub>2</sub>     | +       | 85                               | 219                                       |
| Methanomicrobiales               | <i>Methanogenium marisnigri</i> <sup>1</sup>            | s             | 0.31               | H <sub>2</sub>     | —       | 20                               | —                                         |
|                                  |                                                         |               | 0.24               | H <sub>2</sub>     | +       | 33                               | 9                                         |
|                                  | <i>Methanolobus tindarius</i> <sup>15</sup>             | s             | 0.56               | CH <sub>3</sub> OH | —       | 103                              | —                                         |
|                                  |                                                         |               | 0.57               | CH <sub>3</sub> OH | +       | 87                               | 12                                        |
|                                  | <i>Methanolobus</i> sp. PL-12/M                         | s             | 0.82               | CH <sub>3</sub> OH | —       | 137                              | —                                         |
|                                  |                                                         |               | 0.91               | CH <sub>3</sub> OH | +       | 81                               | 20                                        |
|                                  | <i>Methanoplanus limicola</i> <sup>17</sup>             | s             | 0.13               | H <sub>2</sub>     | —       | 77                               | —                                         |
|                                  |                                                         |               | 0.13               | H <sub>2</sub>     | +       | 70                               | 23                                        |
| Sulfolobales/<br>Thermoproteales | <i>Methanosarcina barkeri</i> <sup>1</sup>              | c             | ND                 | H <sub>2</sub>     | —       | 6                                | —                                         |
|                                  |                                                         |               | ND                 | H <sub>2</sub>     | +       | 5                                | 8                                         |
|                                  |                                                         |               | ND                 | CH <sub>3</sub> OH | —       | 48                               | —                                         |
|                                  |                                                         |               | ND                 | CH <sub>3</sub> OH | +       | 48                               | 9                                         |
|                                  | <i>Pyrodictum occultum</i> <sup>8</sup>                 | vs            | ~0.1               | H <sub>2</sub>     | +       | —                                | 30                                        |

All strains were grown as 20-ml cultures in 100-ml serum bottles using the method described by Balch *et al.*<sup>1</sup>. During the exponential growth phase, 2 g of elemental sulphur were added to each of two parallel cultures, while two others were treated as controls without sulphur. After sulphur addition, the gas phase of all culture vessels was renewed by exchange and the cultures were incubated for a further 55 h. Methane concentration was determined by using a Hewlett Packard gas chromatograph 5880 A equipped with a 6-ft Carbosieve S column and an FID. The oven temperature was 170 °C isothermal. H<sub>2</sub>S concentration was determined by titration<sup>18</sup>. The members of Methanobacteriales and *Methanosarcina barkeri* were grown in medium 1 and all other methanogens in medium 3 (ref. 1) modified by the omission of sodium sulphide. *Pyrodictum* was grown as described elsewhere<sup>8</sup>. The gas phase in cultures of *Methanolobus* and *Methanosarcina* consisted of 80% N<sub>2</sub>, 20% CO<sub>2</sub> (2 bar), and in all other cases, H<sub>2</sub>/CO<sub>2</sub> (at 80:20; 3 bar). Methanol as energy source was added at 1% (v/v). All strains were incubated at optimal growth temperatures. vs, Volcanic submarine sediments; s, submarine sediments; vc, volcanic continental waterholes; c, freshwater sediments. ND, not determined.

\* At the beginning of the experiment.

and in some cases, the amounts of H<sub>2</sub>S produced were well in excess of those produced by obligate sulphur-hydrogen autotrophic organisms (for example, *Pyrodictum occultum*) in the same conditions. The highest H<sub>2</sub>S levels were obtained with the four thermophilic isolates from volcanic habitats. Within the orders Methanobacteriales and Methanococcales, methane formation was greatly lowered in the presence of sulphur, but that by the Methanomicrobiales remained almost unaffected. Among the methanogens, H<sub>2</sub>S was usually formed from H<sub>2</sub> and sulphur, but also from methanol and sulphur, for example by the obligate methylotrophic *Methanolobus*<sup>15</sup> and by *Methanosarcina*.

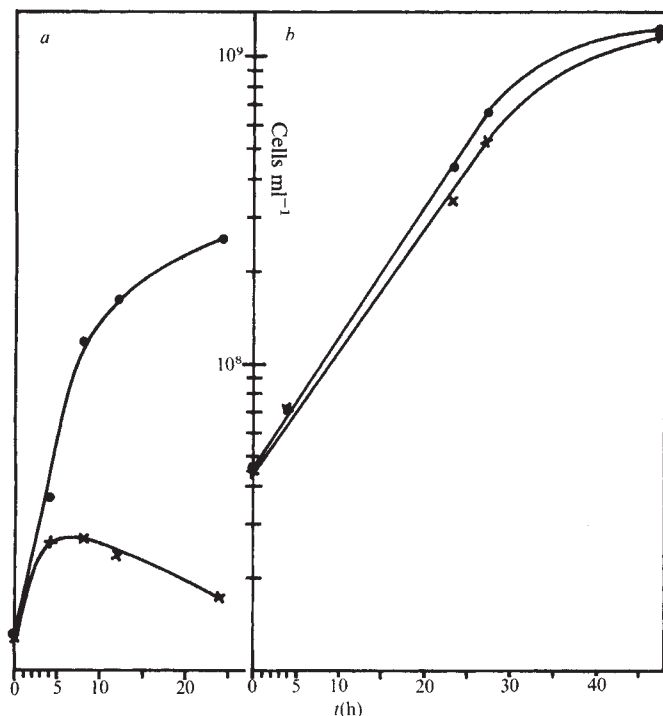
The formation of methane and H<sub>2</sub>S during growth of *Methanococcus thermolithotrophicus* is shown in Fig. 1. In the exponential growth phase, the CH<sub>4</sub>:H<sub>2</sub>S ratio was ~2 in the sulphur-containing culture. At the end of the logarithmic growth phase methanogenesis stopped, while H<sub>2</sub>S formation remained unaffected. After 24 h, cells began to lyse, possibly due to an intoxication by the high H<sub>2</sub>S concentrations which had accumulated during growth in the closed culture vessel. In the control without sulphur, methane production continued also in the stationary growth phase (Fig. 1) and persisted for several days (not shown).

*Methanobacterium* sp. PL-10, which closely resembles *Methanobacterium thermoautotrophicum*<sup>16</sup>, was isolated by us from a sulphurous geothermally heated submarine sediment off Vulcano, Italy. After a short initial growth phase (Fig. 2a), the cells of this organism began to lyse in the presence of sulphur,

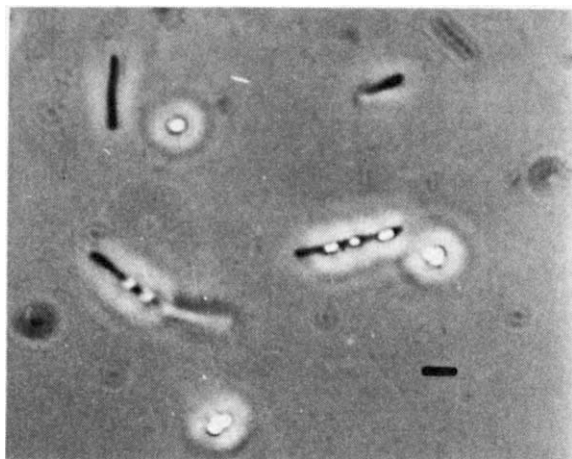
while large amounts of H<sub>2</sub>S were formed (data not shown). The addition of H<sub>2</sub>S (5 μmol ml<sup>-1</sup>) to a non-sulphur-containing culture of the same strain led to growth inhibition (results not shown) indicating that it was caused by H<sub>2</sub>S rather than by sulphur. In nature, H<sub>2</sub>S concentrations may be much lower due to the 'washing-out' effect of the volcanic gases. Sensitivity of growth to H<sub>2</sub>S was found with all other rod-shaped methanogens. After about 5 h in the presence of sulphur, such cells still showed their characteristic UV fluorescence<sup>1</sup>, indicating that they were alive, but they were covered with sulphur granules (Fig. 3). In electron micrographs (not shown) the membranes appeared shrunken as if plasmolysis had occurred during this phase. After 2 days, the rods had lost their UV fluorescence and appeared empty. In contrast, the growth of coccoid *Methanolobus*, and the other members of Methanomicrobiales, remained undisturbed by the H<sub>2</sub>S formed in the presence of sulphur (Fig. 2b).

Compared with methanogenesis, H<sub>2</sub>S formation was strongly favoured by most methanogens in the presence of sulphur. However, some residual methane was formed in all cases, indicating that the switch to H<sub>2</sub>S lithotrophy was incomplete. Methane was formed mainly during the logarithmic growth phase and may be a prerequisite for a sufficient energy yield. However, it cannot be excluded that the culture conditions used may have been inadequate. The natural habitats, for example the volcanic sediments from which some of the methanogens were isolated (Table 1), contained large amounts<sup>14</sup> of sulphur. Furthermore, normal marine sediments also contain sulphur





**Fig. 2** Growth curves in the presence (×) and absence (●) of sulphur. *a*, *Methanobacterium* sp. PL-10 (closely related to *Methanobacterium thermoautotrophicum*) at 65°C. *b*, *Methanobacterium tindarius* at 37°C. Growth conditions were as described in Table 1.



**Fig. 3** Sulphur granules attached to cells of *Methanothermobacter fervidus*. Sulphur was added to an exponentially growing culture of *M. fervidus* which was then incubated at 85°C. The particles could be removed by using carbon disulphide (not shown). Phase contrast micrograph. Scale bar, 1 μm.

(~0.5%; ref. 14). Therefore, H<sub>2</sub>S formation by methanogens should be a common natural phenomenon. Oxygen, which is harmful to these strict anaerobes, can be removed from their biotopes by non-enzymatic reduction of O<sub>2</sub> by H<sub>2</sub>S formed by the bacteria; this may have contributed to the extensive distribution of the methanogens in nature. In the laboratory, these bacteria could be grown readily in sulphur-containing media without removal of oxygen: a few minutes after inoculation, the redox dye resazurin included in the medium became colourless due to reduction of oxygen by the H<sub>2</sub>S formed when the bacteria began to grow (not shown).

Through their ability to reduce sulphur, methanogens are related to the anaerobic sulphur-reducing archaeobacteria, the metabolism of which was thought to be rather primitive<sup>9</sup>. It

remains unclear, however, whether H<sub>2</sub>S formation or methanogenesis was the primeval means of energy conservation during evolution of the archaeobacteria. Possibly, both metabolic systems are related to each other, and methanogenesis could have developed from energetically less efficient sulphide-forming metabolism. Comparison of both metabolic types at the molecular level may shed further light on the evolution of these two groups of organisms.

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## Helical microtubular arrays in onion root hairs

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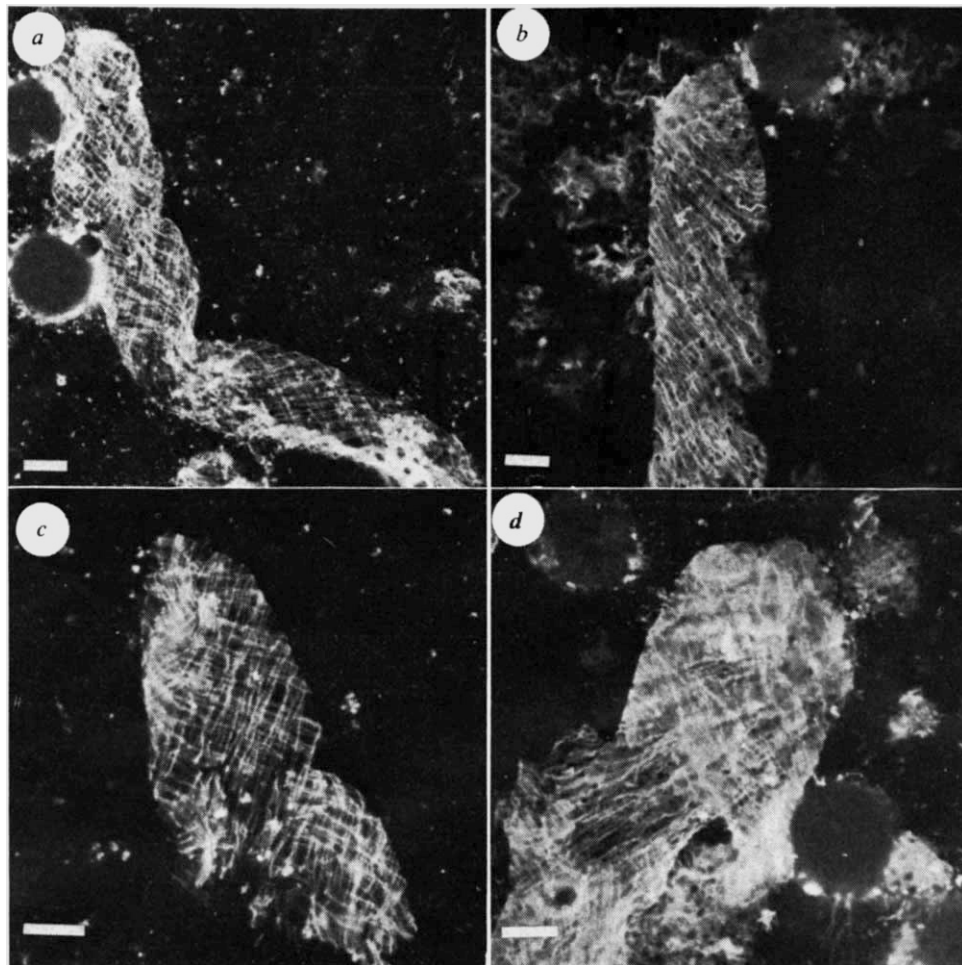
The higher order patterns to which individual plant microtubules contribute are not yet fully described, perhaps because of the problems of reconstructing whole-cell conformations from electron microscope (EM) thin sections. Because microtubules may govern the way in which cellulose microfibrils are deposited in the plant cell wall, it is important to determine the range of permissible conformations for this has implications for the understanding of wall texture. Often, but not always, the EM shows that microtubules occur roughly transversely to the plant cell's long axis and they are usually conceived of as contributing to a series of 'hoops'. However, based on evidence that microtubules can be long relative to the cell's circumference and on the interconnected nature of the cytoskeleton, it was recently proposed that microtubules might be wound helically around the cell; the helical pitch being influenced by the expansion characteristics of the cell. By using whole-cell immunofluorescence and monoclonal antibodies to tubulin, evidence is now presented which confirms that unambiguous helices occur in formaldehyde-fixed onion root hairs.

Interphase microtubule arrays (IMTAs) in glutaraldehyde-fixed plant cells were first described<sup>1</sup> as being "like hundreds of hoops around the cell" although it was recorded that microtubules also diverged from the transverse. This approximately transverse orientation has subsequently been confirmed by numerous investigators<sup>2</sup> but it is now clear that in some cells microtubules may even have a net axial orientation<sup>3</sup> and they can adopt radically different arrangements in response to the way in which the living tissue is treated<sup>4,5</sup>—that is, orientation is varied and mutable.

It has been hypothesized<sup>6</sup> that IMTAs could, in fact, be based on the helix, a possibility prompted by the following observations on carrot cells and protoplasts. First, in fragments of substrate-attached plasma membrane produced by extracting protoplasts with detergent, negatively stained microtubules were detected

**Fig. 1** *a*, This shows the typically continuous nature of the microtubular cytoskeleton of onion root hairs, which here occurs as a right-handed helix. The depth of field is such that in this air-dried cell, the single helix can be clearly seen to wind around both upper and lower surfaces of this compressed cylinder. In *b*, the microtubules are not bundled together but wind around the cell in a denser, sheet-like manner (this time as a left-hand helix). The nucleus is often expelled from its position within tens of micrometres from the tip and in *c* this dislocated tip clearly illustrates the many bundles that wind around the cell towards the tip—all showing a common helical angle. In *d*, the expulsion of the nucleus has exposed a subapical portion of the microtubular structure to reveal microtubules of a single helical sign. This winding does not contain a trellis-work of microtubules of opposite handedness, as would have occurred if microtubules had 'looped-the-loop' at the cell apex. Scale bar, 10  $\mu\text{m}$ .

**Methods:** Onion seeds, *Allium cepa* L. (Bedfordshire Champion), were placed on kitchen paper moistened with tap water and sealed in a deep dish with plastic film. After germinating for 4–5 days in the dark at room temperature, root hairs were fixed by immersing the entire seedling for 50 min in freshly prepared 4% (w/v) formaldehyde in 50 mM phosphate buffer (pH 6.4) containing 5 mM EGTA, according to the method of Wick *et al.*<sup>20</sup>. These were washed for 50 min in phosphate/EGTA and treated for 10 min with 2% (w/v) Onozuka R-10 cellulase (Yakult) in the same buffer. After washing for a further 50 min, the root hairs were removed by scraping the root axis with a razor. Hairs were air-dried onto coverslips that had been previously cleaned with nitric acid and acetone before treatment with 400,000-molecular weight polylysine (1 mg ml<sup>-1</sup>; Sigma). Monoclonal antibody to yeast  $\alpha$ -tubulin (given by Dr J. V. Kilmarin)<sup>21</sup> was then added (25  $\mu\text{g ml}^{-1}$ ) at 37 °C for 60 min. After three 4-min washes, fluorescein isothiocyanate-conjugated goat anti-rat antibody (Miles Yeda) was added at 1:150 dilution and the hairs were incubated at 37 °C for 45 min before washing. Coverslips were mounted in a glycerol buffer containing non-fade additive (Chemistry Department, City University, London), and cells were observed with a Zeiss Universal II microscope, using the  $\times 40$  Planapo objective (NA1.0), and photographs taken using Ilford Pan F film exposed for 30 s.



that were up to 25  $\mu\text{m}$  long (much longer than seen in conventionally fixed, resin-embedded sections) and occasionally formed circular arrays<sup>6,7</sup>. The fact that microtubules in carrot cells<sup>8</sup> associate with the plasma membrane in a layer one tubule deep, encourages the idea that long microtubules (individually or, more probably, with others) wind helically around the cell. Second, by converting elongated, living carrot cells to spherical protoplasts with cellulases<sup>9</sup>, immunofluorescence showed that microtubule hoops could expand to accommodate the change in cell shape (implying that component microtubules somehow move relative to one another) and that even in protoplasts, tubules form cage-type networks without apparent free ends, thus testifying to the integrity of the IMTA and the continuity between the 'hoops'. From this, and by reviewing other evidence<sup>6</sup>, a more general, unifying concept was proposed—that long, overlapping microtubules could form a variety of arrays from 'hoops' (compressed helices) to more steeply pitched forms, the particular morphology depending on the expansion characteristics of the cell. Clearly, it is not intended to suggest that all IMTAs will be seen as uncomplicated helices but if the above ideas are correct it should be possible to demonstrate this arrangement in certain favourable cases.

In the present study, onion root hairs were used; they are freely growing and tubular and by using monoclonal antibody to yeast  $\alpha$ -tubulin, it is possible to demonstrate the helical

arrangement of microtubules in these fixed cells. Generally, the microtubules formed either left- or right-handed helices of  $\sim 45^\circ$  pitch. The helices were continuous over the entire length of the cell, even around the flared base of the cell, when present. Microtubules were present at the tip, filling the apical dome, in contrast to reports on radish root hairs<sup>3,10</sup> where tubules were not found to invade the tip extensively. It was not possible to decipher the behaviour of the IMTAs at the tip but in Fig. 1*d*, the cell is broken open sub-apically to reveal a sheet-like winding. This illustrates the general observation that these multi-start helices are, within a single surface, either right- or left-handed but not both as would be expected if tubules had 'looped-the-loop' at the tip to initiate a helix of opposite sign (see ref. 6).

Green<sup>11</sup> has proposed that circumferential microtubules form a contracting ring to regulate the diameter of cylindrical cells. The helices illustrated here do not necessarily negate that idea as root hairs are assumed to exhibit tip growth and therefore to elongate in a manner dissimilar to that of cells exhibiting a more uniform expansion. The composition of the wall and the mode of its deposition may, therefore, be different<sup>12</sup> in these two classes of cells and thus the biophysical constraints under which IMTAs form may be sufficiently different as to encourage the expression of different conformations of microtubules. Nevertheless, the present observations illustrate that microtubules can form stable assemblies that are not based on circles.



It is difficult to know how the helical IMTAs originate, for the freely growing habit of root hairs probably places them outside the scope of present ideas concerning cells constrained in tissues. One suggestion, based on observations of root cells in the water fern *Azolla*<sup>13</sup>, is that microtubule organizing centres (thought to be inherited along new cell edges following cytokinesis) initiate microtubules from opposing cell edges such that they inter-digitate across each cell face and eventually encircle the cell. Cells, such as hairs, that are without edges and corners in the long axis, and where that long axis is produced by outgrowth rather than by cytokinesis, raise difficulties for this notion, as Busby and Gunning<sup>14</sup> have discussed. An alternative hypothesis<sup>6</sup> is that helices necessarily result from the way in which long elements more than circumnavigate the cell while associating with the plasma membrane.

A major interest in the role of microtubules in plant morphogenesis results from the fact that they mirror patterns of cellulose deposition and may therefore help to determine cell shape; some of the early ideas on wall patterns derived from

hairs and other freely extending cells<sup>15,16</sup>. It is interesting, therefore, that the innermost layer of cellulose microfibrils is reported in these and other studies as being helically wound. More recent studies<sup>17</sup> on a variety of root hairs also indicate that some cells have helical inner wall layers. In addition, in cotton fibres, the helical wall is underlain by microtubules that remain parallel to the cellulose microfibrils even while they reverse gyre<sup>18</sup>. An equally remarkable congruence between helical wall microfibrils (in this case, chitin) and cytoplasmic microtubules has been described by Schnepf *et al.*<sup>19</sup> for the alga, *Poterioochromonas stipitata*.

The relationship between the orientation of microtubules and of nascent microfibrils is not yet fully understood but if the former elements are accepted as having a guiding role, it is reasonable to suppose that patterns of microfibrillar deposition will not be infinitely various but limited to the patterns that microtubules are capable of forming. The present results demonstrate for higher plants that the helix is one such pattern.

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## Differential inhibition by erythro-9-[3-(2-hydroxynonyl)]adenine of flagella-like and cilia-like movement of *Leishmania* promastigotes

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**Erythro-9-[3-(2-hydroxynonyl)]adenine (EHNA) inhibits axonemal dynein ATPase activity and hence the beating of sea urchin and mammalian flagella<sup>1,2</sup>. We have found that EHNA has an unusual effect on the flagella of *Leishmania* promastigotes in that it alters both the waveform and polarity of the beat. We report here results which suggest that in *Leishmania* promastigotes there are either distinct EHNA-sensitive dyneins or different conformational states of a single dynein involved in the cilia-like and flagella-like waveforms and in the propagation of flagellar waves from tip-to-base and from base-to-tip.**

Trypanosomatids are unusual in that the flagellar wave is normally propagated proximally from the tip<sup>3–5</sup>. In particular, the flagellar wave of *Leishmania* promastigotes is initiated at the tip, and the cell is propelled forwards in the direction of the flagellum (Fig. 1, frames 1–27). However, these forward movements are interspersed by sudden changes in direction caused by several (usually five) eccentric cilia-like beats (Fig. 1, frames 28–36, 36–45, 45–56, 56–74 and 74–91), each of which has the effect of rotating the animal by 10–30°. The wave of this cilia-like eccentric beat is initiated from the base and is propagated from base-to-tip, with an effective stroke followed by a recovery stroke.

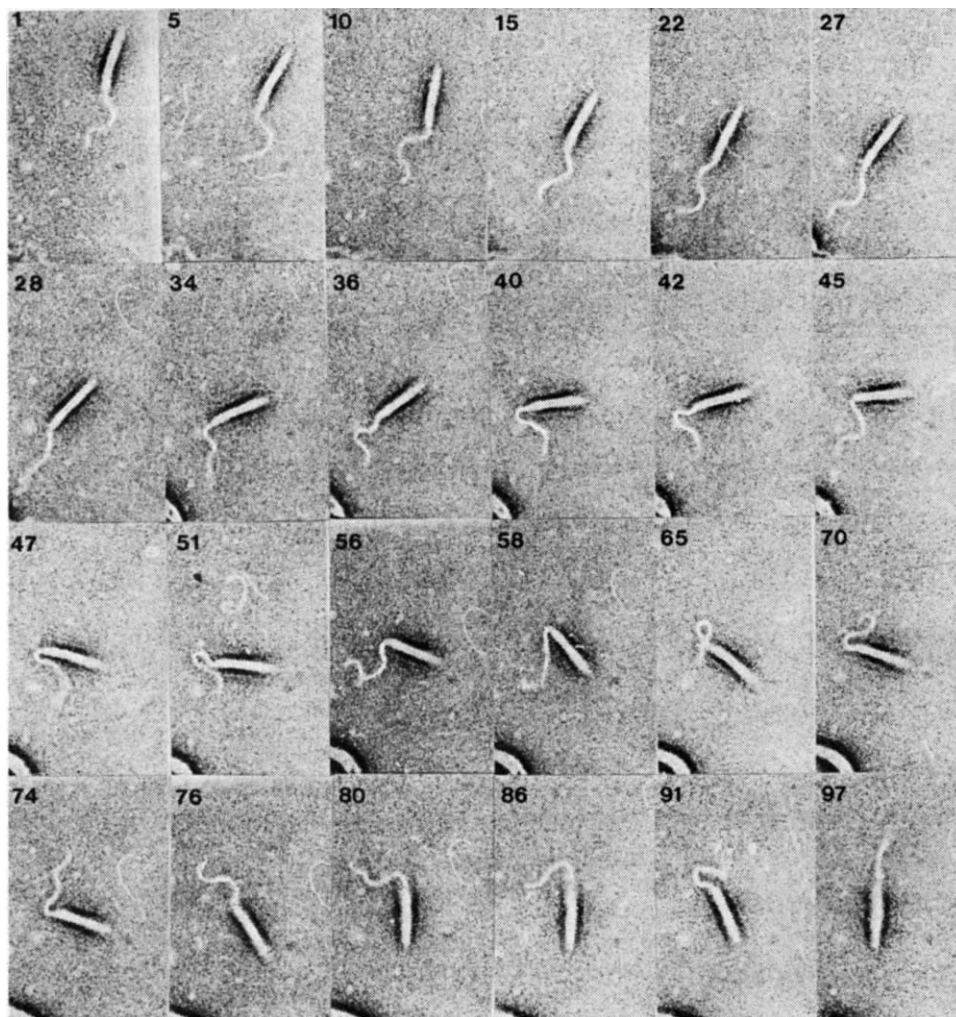
We have observed tip-to-base flagellar and base-to-tip cilia-like beating in several *Leishmania* species, including *Leishmania mexicana mexicana*, *Leishmania major*, *Leishmania donovani*, *Leishmania enriettii* and *Leishmania braziliensis guyanensis*. Typically, the promastigotes move rapidly at 60–120  $\mu\text{m s}^{-1}$ ,

with the flagella alternating between the flagella-like tip-to-base and the cilia-like base-to-tip waveform. This alternating pattern of beating results in movement reminiscent of the 'run and tumble' activity of flagellated bacteria which allow the bacteria to respond to a chemical gradient<sup>6</sup>. Indeed, *Leishmania* promastigotes also migrate along chemical gradients<sup>7</sup>, and they achieve this by changing the frequency at which they switch between the flagella-like and cilia-like waveforms (our unpublished data).

We have observed that increasing concentrations of EHNA have a remarkable effect on the motility of *L. m. mexicana* promastigotes (Table 1). High concentrations of EHNA (9–10 mM) caused an immediate and irreversible inhibition of all flagellar movement. By contrast, treatment with 7–8 mM EHNA caused a rapid loss of motility, but removal of the EHNA within 20 min restored only the flagellar tip-to-base waveform; the eccentric cilia-like waveform was totally and irreversibly inhibited. At 4–6 mM EHNA, both the normal flagellar tip-to-base motility and the eccentric cilia-like beating were totally inhibited, but prompt removal of the EHNA had a remarkable effect. The sinusoidal tip-to-base waveform was restored, whereas the base-to-tip waveform, which is normally cilia-like, adopted a sinusoidal waveform. In these conditions, there are two distinct sinusoidal waveforms travelling along the flagellum, the normal one from tip-to-base and a latent one from base-to-tip, and as the waves pass each other the flagellum appears frozen. The two sinusoidal waveforms are also apparent for EHNA concentrations of 1.5–2.5 mM, conditions which inhibit the base-to-tip cilia-like whiplash but do not affect the normal tip-to-base sinusoidal waveform.

Clearly, there are three distinct forms of motility distinguished by their polarity and waveform which can be selectively inhibited by EHNA.

As intact cells were used in this study, the differential EHNA-sensitivity might be due to a selective effect of EHNA on the axonemal membrane. Unfortunately, we have so far been unable to reactivate demembranated *Leishmania* flagella. However, it seems unlikely that the differential sensitivities of the three forms of flagellar movement simply result from poor penetration of EHNA; indeed, the multiple effects indicate that EHNA binds



**Fig. 1** Movement of a free-swimming *L. m. mexicana* promastigote exhibiting the tip-to-base flagellar waveform and, intermittently, the cilia-like base-to-tip waveform. The cell is being propelled forward ( $70 \mu\text{m s}^{-1}$ ) by the sinusoidal flagellar beat (frames 1–27) at which time it is rotated through  $\sim 180^\circ$  by five consecutive cilia-like beats (frames 28–36, 36–45, 45–46, 56–74 and 79–41). Cells were grown in 4% blood agar No. 2 (Oxoid)–10% defibrinated rabbit blood, with Hank's balanced salts overlay. Immediately before study, they were washed twice in Hank's balanced salt medium containing 1% (w/v) glucose, then incubated in the same medium supplemented with the appropriate concentration of EHNA. Movement was observed using a Leitz Orthoplan microscope ( $\times 500$ ), and recorded on either double-negative film (50 frames  $\text{s}^{-1}$ , as shown) using a Bolex camera, or on video film.

to more than one non-equivalent acceptor. However, although EHNA is known to inhibit both dynein ATPase and carboxymethylase activities<sup>1</sup>, other inhibitors of the carboxymethylase (0.5–2.0 mM adenosine or 10 mM cycloleucine) have no detectable effect. It is probable, therefore, that the effects of EHNA on *Leishmania* flagella are due to the inhibition of axonemal dynein ATPase activity. One possible alternative is that EHNA inhibits the ATPase of the paraflagellar rod, as this rod has been shown in *Euglena* to have an ATPase activity which is unrelated to the dynein ATPase of the axoneme<sup>8</sup>. However, the free-swimming *Crithidia oncopelti*, which lacks a paraflagellar rod, can also generate both sinusoidal and eccentric beats from the base of the flagellum<sup>5</sup>, indicating that these two forms of motility are not correlated with the presence of the paraflagellar rod.

The differential inhibition reported here suggests that the effects are mediated through distinct, EHNA-sensitive dynein ATPases, or a single dynein existing in several conformational states, each specifying the polarity and waveform of the flagellar beat. The outer dynein-1 sidearm of sea urchin axonemes is highly complex with two high-molecular weight polypeptides, three intermediate-sized polypeptides, and four additional light chains<sup>9</sup>, so that the possible conformational states may represent differing interactions within this aggregate. The proposal of multiple dyneins, or dynein conformational states, is consistent with recent observations that the polarity of the flagellar wave of *Crithidia* can be reversed by inhibitors of calmodulin<sup>10</sup>, and by earlier evidence that the free calcium concentration modulates the waveform<sup>11</sup>, as the specific dynein activities may be

**Table 1** Effect of EHNA on mobility of *L. m. mexicana* promastigotes

| EHNA concentration | Polarity of flagellar beat |               |                         |             |               |                         |
|--------------------|----------------------------|---------------|-------------------------|-------------|---------------|-------------------------|
|                    | Tip-to-base                |               |                         | Base-to-tip |               |                         |
|                    | Waveform                   | Reversibility | Waveform after reversal | Waveform    | Reversibility | Waveform after reversal |
| Control (0 mM)     | Sinusoidal                 |               |                         | Cilia-like  |               |                         |
| 1.5–2.5 mM         | Sinusoidal                 |               |                         | Sinusoidal  | –             |                         |
| 4–6 mM             | –                          | +             | Sinusoidal              | –           | +             | Sinusoidal              |
| 7–8 mM             | –                          | +             | Sinusoidal              | –           | –             | –                       |
| 9–10 mM            | –                          | –             | –                       | –           | –             | –                       |

For waveform, – indicates that flagellar beating was totally inhibited. For reversibility, + indicates that beating was restored after removal of the EHNA by diluting the EHNA-treated promastigotes 10-fold with Hank's balanced medium–1% (w/v) glucose, pelleting the cells at 300g, and resuspending in EHNA-free medium.



independently controlled. It is possible that the molecular mechanism involved in the generation of the three types of flagellar movement binds both EHNA and calmodulin. It is unfortunate that the *Leishmania* axoneme cannot be reactivated, as this precludes a direct test of whether EHNA and  $\text{Ca}^{2+}$  calmodulin inhibit a common mechanism.

Our results suggest that EHNA may prove useful in identifying which of the dyneins, or dynein conformational states, are responsible for each of the flagella-like and cilia-like waveforms of *Leishmania* promastigotes. Similarly, EHNA-inhibited cytoplasmic movement, such as anaphase B<sup>12</sup> and granule translocation in chromatophores<sup>13</sup>, may also exhibit differential EHNA sensitivities.

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## Monensin inhibits initial spreading of cultured human fibroblasts

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**The monovalent ionophore, monensin, inhibits the secretion of both pro-collagen and fibronectin in cultured human fibroblasts<sup>1–3</sup> and other cell types<sup>4–7</sup>. The block to secretion is due to the ability of monensin to suppress the export of these secretory proteins from the Golgi apparatus<sup>3,8,9</sup>. As such proteins are known to be implicated in the adhesion, spreading and movement of cultured fibroblasts<sup>10,11</sup>, it might be expected that monensin treatment would interfere with these processes. However, it has recently been reported<sup>9</sup> that monensin-treated human embryonal fibroblasts attached and spread onto glass substrata to the same extent as untreated cells, although at later stages they fail to develop focal adhesion sites. However, these experiments were performed using medium supplemented with fetal calf serum (FCS). We now demonstrate that in the absence of FCS, while monensin has little or no effect on the initial adhesion of fibroblasts to the substratum, subsequent spreading is much reduced. The inhibition of spreading is noticeable within 30 min of plating and is maintained for at least 100 min in monensin-free medium following prolonged pre-incubation of the cells with monensin.**

Cultured human skin fibroblasts were plated after trypsinization onto clean glass in serum-free medium and allowed to spread for periods of up to 100 min at 37 °C. Initial seeding efficiency was estimated to be >90% in all cases. Within 10 min of plating, cells had attached to the substratum but had not yet begun to spread. By 30 min in culture, almost all cells appeared with a narrow skirt of cytoplasm extended onto the substratum and at 60 min most cells had spread extensively, giving rise to the characteristic 'fried egg' morphology of rapidly spreading fibroblasts. By 100 min, the rate of spreading had decreased, and at later stages many cells had become polarized with promi-

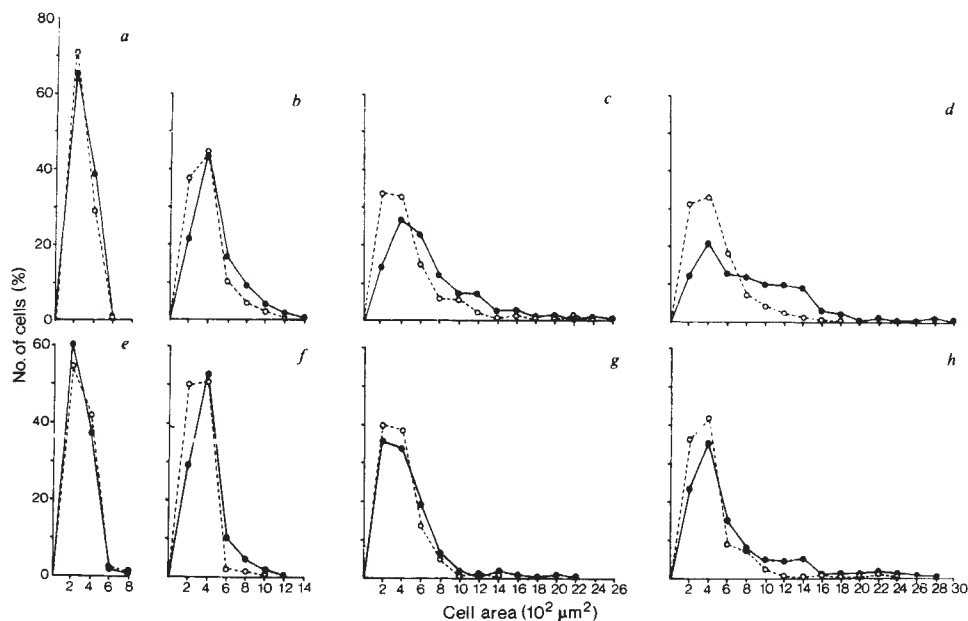
nent retraction fibres, indicating that the cells were locomoting rather than spreading.

When monensin ( $5 \times 10^{-7}$  M) was added to the medium immediately before seeding of the cell suspension, cell spreading was consistently inhibited. After 10 min, there were no obvious differences in size or morphology between monensin-treated and control cells. However, by 30 min most monensin-treated cells had clearly spread less than untreated controls and this effect was maintained for the duration of the experiment. No difference was found in the seeding efficiency of monensin-treated compared with untreated cells and no detachment of the monensin-treated cells from the substratum was observed during the time course of the experiments. Fig. 1*a–d* shows the rate of spreading of control and monensin-treated cells; treated cells (open circles) had spread maximally by 60 min. At this stage, control cells (closed circles) were still spreading, as shown by the progressive shift of the cell area frequency distributions to the right in Fig. 1*a–d*. Comparison between the area distributions of control and monensin-treated cells was performed using the Kolmogorov–Smirnov two-sample test<sup>12</sup> as, once the cells have started to spread, the measured cell areas fit a non-gaussian distribution (Fig. 1*b–d*). This analysis showed significant differences ( $P < 0.01$ ) between the area distributions of monensin-treated and control fibroblasts by 30 min of spreading (Fig. 1*b*).

In a further series of experiments designed to investigate the recovery from long-term exposure to monensin, subconfluent cultures of human fibroblasts, with an established fibronectin matrix, were incubated for 20 h with  $5 \times 10^{-7}$  M monensin before trypsinization. Following dissociation, cells were plated and allowed to spread for periods up to 100 min in the presence or absence of the ionophore. Spreading was greatly reduced in both groups of cells compared with untreated controls (Fig. 1*e–h*), although removal of the monensin block before plating (Fig. 1*e–h*, closed circles) did result in some recovery of spreading ability. This was noticeable by 30 min ( $P < 0.01$ ) as determined by comparison of the cell area frequency distributions (Fig. 1*f*). By 100 min, the extent of spreading of monensin-released cells (Fig. 1*h*, ●), although much inhibited, was greater ( $P < 0.01$ ) than that of cells only exposed to monensin during spreading (Fig. 1*d*, ○). These data indicate that the secretory system may not be permanently disabled by long-term exposure to monensin.

These findings demonstrate that although monensin has little or no effect on initial cell attachment (or subsequent detachment), the ionophore does suppress the early spreading of human fibroblasts. The quantitative data, obtained using the semi-automated Kontron MOPPET system<sup>13</sup>, are in contrast to the observation of Virtanen *et al.*<sup>9</sup> who suggested that monensin does not affect fibroblast spreading and that this is evidence of a lack of requirement for fibronectin in spreading. Our results suggest that fibronectin might be needed to promote initial cell spreading and it has been shown, using measuring techniques similar to our own, that human fibroblasts do spread more rapidly on fibronectin-coated and human serum-coated substrata<sup>14</sup>. The time course of the appearance of freshly secreted fibronectin at the surface of cells is not yet fully resolved. Grinnell and Feld<sup>11</sup> have identified fibronectin, by immunofluorescence, on the surfaces of human fibroblasts by only 10 min after plating. However, others<sup>9</sup>, including ourselves (unpublished data), can confirm the presence of surface-located fibronectin only after a lag of 3–5 h using the same techniques. In a series of pulse-chase experiments, Uchida *et al.*<sup>2</sup> detected the rapid appearance of newly secreted pro-collagen and fibronectin in the culture medium of human fibroblasts and also presented data which showed that monensin does not totally inhibit secretion of these proteins. Monensin appears to halve the secretion rate and, on removal of the monensin block, there follows a lag period after which secretion returns to the rate found in untreated fibroblasts<sup>3</sup>. These results are in agreement with those presented here, where the rate of cell spreading was found to increase within 30 min of release from a prolonged monensin block.

**Fig. 1** Frequency distributions of monensin-treated and control cell areas. *a-d* Shows the effect of the addition of monensin to the cell suspension immediately before plating: ●, untreated cells; ○, monensin-treated cells. Area distributions are shown for cells which were fixed 10 (*a*), 30 (*b*), 60 (*c*) and 100 (*d*) min after plating. The area distribution of monensin-treated fibroblasts is significantly different ( $P < 0.01$ ) from the controls at 30 min (*b*), and at 60 min inhibition of spreading in the treated cells is complete. There is no significant difference between the area distributions of the treated cells in *c* and *d*. *e-h*. The effect of monensin pre-incubation on fibroblast spreading. Area distributions are shown for monensin-block-maintained (○) and monensin-block-released (●) cells which were fixed 10 (*e*), 30 (*f*), 60 (*g*) and 100 (*h*) min after plating in MEM. In all cases except *e* (which is the area distribution of attached but unspread cells), the frequency distributions of the monensin-block-maintained cells (○ in *e-h*) are skewed further to the left of those at the same time point which were not exposed to monensin at any stage (● in *a-d*). However, by 100 min, monensin-block-released cells (*h*, ●) have spread to a greater extent than those in which the monensin block was maintained (*h*, ○). This causes the area distribution of the former population to be skewed further to the right and to be significantly different ( $P < 0.01$ ) from the monensin-block-maintained cells.



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**Methods:** Cultured human skin fibroblasts were established from explants of skin biopsies from healthy volunteers and were used between passages 8 and 20, as described previously<sup>17</sup>. All cultures were maintained at 37 °C, grown to near-confluency and then incubated at room temperature in 0.25% trypsin in Ca<sup>2+</sup>- and Mg<sup>2+</sup>-free Hank's basal salt solution (HBSS) for 20 min. Cells were centrifuged at 150g for 5 min at 4 °C, washed in HBSS, and resuspended in MEM to a final density of  $2 \times 10^5$  cells ml<sup>-1</sup>. Cells from each flask were divided into two aliquots and allowed to attach and spread in MEM containing either  $5 \times 10^{-7}$  M monensin (Calbiochem) in ethanol, or 0.1% ethanol alone (monensin control) at 37 °C for periods of up to 100 min on clean glass multitest slides (Flow Laboratories). Cell spreading was stopped by washing briefly in 0.1 M sodium cacodylate buffer (pH 7.2), followed by fixation in 2.5% glutaraldehyde in cacodylate buffer. Cells were examined using differential interference microscopy. Orthogonal diameters were drawn randomly across the slides and, from each of the cultures used, 50 cells which fell on or near these diameters were photographed. Measurements of cell areas were then made from prints using the MOPPET system described previously<sup>13</sup>. Each point on the graph represents the mean of the subsets analysed and the distributions were compared using all the cells measured (300 in each case) by the Kolmogorov-Smirnov two-sample test<sup>12</sup>, which is sensitive to differences, either of form or location, between the two populations. For both monensin-treated and control cells (*a-d*), the mean distribution of six subsets of 50 cells is shown. In *e-h*, three cultures were incubated with  $5 \times 10^{-7}$  M monensin for 20 h before trypsinization and the cell suspensions were split before seeding the cells in MEM. In each case, monensin was re-introduced into one aliquot (○) and 0.1% ethanol was added to the other (●). Cells were allowed to spread at 37 °C as described above. Means of the subsets were taken and the distributions were compared as before.

As monensin affects all Golgi-associated secretion our results cannot be taken as rigorously identifying a specific requirement for fibronectin in cell spreading. However, on the basis of monensin-block experiments, it has been suggested<sup>9</sup> that the early stages of cell spreading do not require such secreted proteins, specifically fibronectin. Our data do not support this suggestion and the possibility remains that fibronectin is required in the early stages of fibroblast spreading on a substratum. The results discussed by Virtanen *et al.*<sup>9</sup> are based on a subjective, visual estimation of spreading in cultures following 5 h of plating in the presence of serum. However, it is well established that serum contains sufficient fibronectin to promote spreading in cells that produce low levels of endogenous fibronectin<sup>15</sup>. For this reason, together with the finding that suppression of fibronectin secretion by monensin is only partial<sup>3</sup>, the quantitative data reported here were obtained in serum-free media and during the early stages of the spreading process. We have found (unpublished data) that 60 min after seeding in minimal essential medium (MEM) supplemented with 10% FCS, the mean area of control and monensin-treated cells was not significantly different. In these conditions, while both control and monensin-treated fibroblasts spread considerably more than in serum-free media (270% and 285%, respectively), monensin-treated cells were indistinguishable from the controls with respect to morphology and any of the statistical parameters of cell area distributions. Thus, it seems that plasma fibronectin in serum is sufficient to mask the very real effect of monensin on early fibroblast spreading via its suppression of endogenous fibronectin secretion.

Finally, it is of interest that focal adhesion sites do not appear to develop in fibroblasts following prolonged incubation with monensin<sup>3</sup>. Recently, it has been shown<sup>16</sup> that fibroblasts secrete an incompletely glycosylated form of fibronectin during prolonged exposure to the ionophore; this may affect the biological properties of the glycoprotein. It is possible that failure of focal contact and vinculin plaque formation following prolonged monensin exposure, is due to the secretion of incompletely processed fibronectin. Further work will be required to examine this and other possible explanations, but the findings of Ledger *et al.*<sup>16</sup> may prove important in determining the mechanisms of cell-substrate interactions and the role of fibronectin in these mechanisms.

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## Diacylglycerol and phorbol ester stimulate secretion without raising cytoplasmic free calcium in human platelets

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An increase in cytoplasmic free calcium,  $[Ca^{2+}]_i$ , is thought to be the trigger for secretory exocytosis in many cells<sup>1–3</sup>. In blood platelets, large rises in  $[Ca^{2+}]_i$  can cause secretion<sup>4,5</sup> and calcium has been regarded as the final common activator not only for secretion but also for shape-change and aggregation<sup>6,7</sup>. We have shown that while thrombin<sup>8</sup> and platelet-activating factor (PAF)<sup>9</sup> normally elevate  $[Ca^{2+}]_i$ , they can also stimulate shape-change and secretion even when the  $[Ca^{2+}]_i$  rise is suppressed. The present results strongly implicate diacylglycerol, produced by stimulus-dependent breakdown of phosphoinositide<sup>9–12</sup>, in this calcium-independent activation. Exogenous diacylglycerol activates a protein kinase (C-kinase) in platelets as do PAF, thrombin and collagen. 12-*O*-tetradecanoyl phorbol-13-acetate (TPA) also activates C-kinase<sup>13</sup> and is a potent stimulus for secretion and aggregation<sup>14–16</sup>. It is shown here that the exogenous diacylglycerol 1-oleoyl-2-acetyl-glycerol (OAG) and TPA evoke similar secretion and aggregation without elevating  $[Ca^{2+}]_i$  above the basal level of 0.1  $\mu$ M. The pattern of secretion resembles that produced by collagen and thrombin when  $[Ca^{2+}]_i$  remains at basal levels. Modest increases in  $[Ca^{2+}]_i$ , insufficient to stimulate secretion, markedly accelerate the responses to TPA and OAG.

The methods for incorporating quin2 into intact cells and using its fluorescence to monitor  $[Ca^{2+}]_i$  have been detailed elsewhere, together with evidence that the trapped indicator is largely confined to the cytoplasm and measures cytoplasmic free calcium<sup>17,18</sup>. Shape-change and aggregation were monitored by the standard technique of recording absorbance (*A*) of a stirred suspension<sup>19</sup>. Sometimes the absorbance was monitored simultaneously with quin2 fluorescence in a purpose-built cuvette holder, but more often it was measured in an aliquot of the same preparation of quin2-loaded cells in a Chronolog 'lumi-aggregometer' which permits on-line recording of released ATP as a measure of exocytotic secretion of the contents of dense granules. In some experiments,  $\alpha$ -granule secretion was monitored by radioimmunoassay (Amersham) of released  $\beta$ -thromboglobulin.

Figure 1*a–c* shows responses to an optimal concentration of TPA:  $[Ca^{2+}]_i$  was not detectably elevated<sup>17,20</sup> but there was secretion of ATP. The secretion was unaffected by removal of external calcium or treatment with aspirin which blocks production of prostaglandin endoperoxides and thromboxane A<sub>2</sub>. Although there was a lag after application of TPA and secretion was slow, it was substantial. In medium containing 1 mM  $Ca^{2+}$ , the ATP release, expressed as percentage ( $\pm$ s.e.) of that releasable by thrombin, was  $77 \pm 2.5\%$  ( $n = 16$ ) and in calcium-free medium  $78 \pm 3\%$  ( $n = 10$ ). In cells treated with 100  $\mu$ M aspirin or 10  $\mu$ M indomethacin, the values were  $79 \pm 4.4\%$  ( $n = 5$ ) and  $71 \pm 2.5\%$  ( $n = 14$ ). Figure 1*a* shows that with 1 mM external calcium the platelets aggregated in a manner similar to that found previously for platelet-rich plasma<sup>14,15</sup>. There was no aggregation in the absence of external calcium, presumably because calcium is required for cell–cell attachment. The absorbance traces show a loss of noisiness following addition of TPA, again resembling previous findings<sup>15</sup>. This may indicate a partial

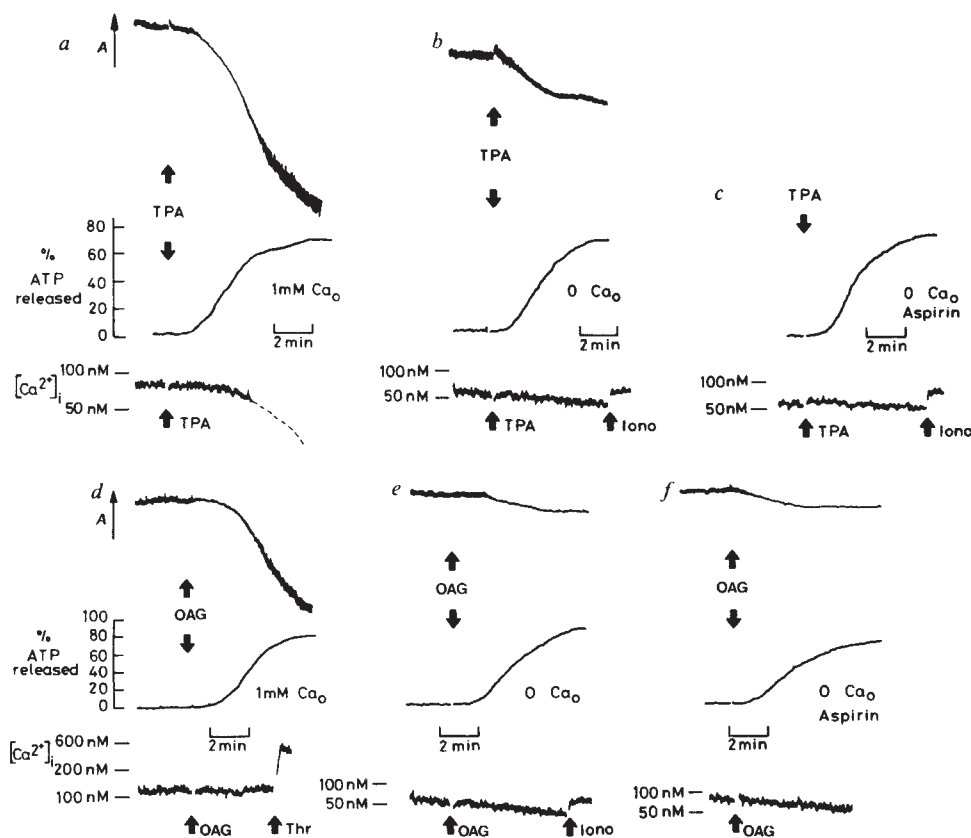
shape-change with disk-to-sphere transition but not pseudopod formation<sup>15</sup>. The fluorescence traces show that TPA did not elevate  $[Ca^{2+}]_i$  beyond the initial level of 85 nM in the 1 mM calcium medium, or 60 nM in the calcium-free medium. (Figure 1*a* in fact shows a declining signal after 3–4 min which is due to the formation of aggregates that interfere with the fluorescence. If the cuvette is not stirred, aggregates form much more slowly and the trace remains stable for many minutes. Secretion was not reduced by lack of continuous stirring, as shown in Figs. 1*c* and 3*a*.) It has been suggested<sup>21,22</sup> that TPA might work partly by mobilizing cellular calcium. The present results appear to rule out such an effect in human platelets, which do possess a releasable pool of internal calcium. Figure 1*b, c* shows that ionomycin, a calcium ionophore, can release this pool when added after TPA. The increase in  $[Ca^{2+}]_i$  evoked by ionomycin was similar to that observed in these cells before application of TPA, and similar to that previously observed in similarly prepared cells<sup>5</sup>.

Figure 1*d–f* shows the very similar pattern of responses produced by OAG. (This diacylglycerol has been used as it is effective when added to cell suspensions. It has been shown to activate C-kinase *in vitro* and to promote phosphorylation of the 40,000-molecular weight substrate in intact platelets<sup>11</sup>. More hydrophobic diglycerides, such as the natural products of phospholipase C activity, are apparently too hydrophobic to reach the relevant site when added to cells in suspension.) There was substantial slow secretion of ATP, which was not dependent on external calcium or thromboxane A<sub>2</sub> production. In the experimental conditions of Fig. 1, OAG gave  $57 \pm 4\%$  ( $n = 10$ ) ATP release in 1 mM calcium medium,  $62 \pm 3\%$  ( $n = 8$ ) in calcium-free medium. In aspirin-treated cells the values were  $66 \pm 4\%$  ( $n = 12$ ) in 1 mM calcium medium and  $64 \pm 4\%$  ( $n = 11$ ) in calcium-free medium. In none of the conditions tested did OAG give a significant elevation of the measured  $[Ca^{2+}]_i$ . As with TPA, there was aggregation in the presence of external calcium and an indication of disk-to-sphere transition independent of external calcium.

The ATP release induced by TPA and OAG seemed to be due to exocytosis of dense granules. It has previously been shown that TPA releases the contents of dense granules without releasing cytoplasmic proteins and in our experiments the absorbance traces and retention of quin2 exclude cell disruption or generalized leakiness. We also found that TPA and OAG both stimulated secretion of  $\beta$ -thromboglobulin. In 1 mM calcium medium, TPA caused release of  $2.9 \pm 0.17$  ( $n = 6$ )  $\mu$ g per  $10^8$  platelets and OAG  $2.7 \pm 0.16$  ( $n = 10$ )  $\mu$ g per  $10^8$  platelets. With aspirin-treated cells in calcium-free medium, the values for TPA were  $2.7 \pm 0.13$  ( $n = 7$ ) and for OAG  $2.7 \pm 0.14$  ( $n = 12$ )  $\mu$ g per  $10^8$  platelets. The maximum  $\beta$ -thromboglobulin released by thrombin was  $2.8 \pm 0.14$  ( $n = 7$ ) and the total content measured in sonicated preparations was  $3.1 \pm 0.07$  ( $n = 6$ )  $\mu$ g per  $10^8$  platelets. These results demonstrate that secretion of  $\alpha$ -granule contents can also be triggered independently of changing  $[Ca^{2+}]_i$ .

The above results show that TPA and externally applied OAG can stimulate secretion without causing or requiring elevation of  $[Ca^{2+}]_i$ . Diacylglycerol is known to be formed as a result of phosphoinositide turnover in response to thrombin, collagen and PAF<sup>9–12,23</sup>, and is thus a strong candidate as an intracellular trigger for the secretion that these agonists can evoke, while  $[Ca^{2+}]_i$  remains at or near basal levels. Collagen produces, in aspirin-treated quin2-loaded platelets, a secretory response with a time course resembling that seen with OAG or TPA, and with no measurable change in  $[Ca^{2+}]_i$  (Fig. 2*a*). Figure 2*b* shows the secretion produced by thrombin in conditions where  $[Ca^{2+}]_i$  does not exceed the normal basal level; in these conditions (see Fig. 2 legend), the initial  $[Ca^{2+}]_i$  was 60 nM and although thrombin did cause a discharge of residual internal calcium stores, the actual rise in  $[Ca^{2+}]_i$  was small and  $[Ca^{2+}]_i$  did not exceed 100 nM, and thus remained far below the micromolar level needed for  $[Ca^{2+}]_i$  itself to initiate secretion. Again, the secretory response has a delay and slow time course resembling that

**Fig. 1** Responses of quin2-loaded human platelets to TPA and OAG. The upper trace of each set of three shows the absorbance, the middle the ATP released and the lower trace  $[Ca^{2+}]_i$  measured in parallel from the quin2 fluorescence in the same batch of cells. *a-c* Show the effects of 20 nM TPA (Sigma) added from dimethyl sulphoxide (DMSO) stock solution, in the presence or absence of external  $Ca$  as indicated, or pre-treatment with 100  $\mu$ M aspirin as indicated. (This concentration of aspirin completely blocked the secretion and aggregation normally produced by 10  $\mu$ M arachidonate.) *d-f* Show similarly the effects of 60  $\mu$ g ml<sup>-1</sup> OAG from 60 mg ml<sup>-1</sup> stock. In 1 mM  $Ca$ , human thrombin (Calbiochem) added after OAG gave a marked rise in signal (which was cut short by the formation of aggregates), showing that the presence of OAG had not prevented quin2 reporting  $[Ca^{2+}]_i$ ; similarly, in *e*, ionomycin (Iono) produced the expected small elevation of fluorescence, presumably due to release of internal  $Ca$ <sup>5</sup>. The quin2 fluorescence in *a-c* was recorded without continuous stirring, and in *d-f* with continuous stirring. In *c*, the lumi-aggregometer stirrer was turned off. The platelets were prepared from freshly drawn blood taken into acid-citrate-dextrose anticoagulant, and centrifuged at 700g for 5 min. The platelet-rich plasma was incubated with 15  $\mu$ M quin2 acetoxymethyl ester at 37 °C, and the cells now loaded with ~1 mM quin2, were centrifuged for 15 min at 350g and suspended in a physiological saline containing 145 mM NaCl, 5 mM KCl, 1 mM MgSO<sub>4</sub>, 10 mM Na-HEPES, 10 mM dextrose, pH 7.4 at 37 °C. Hirudin (0.05 U ml<sup>-1</sup>; Sigma) was then added to prevent activation by any residual traces of thrombin. 1 mM CaCl<sub>2</sub> or 1 mM Na<sub>2</sub>H<sub>2</sub>EGTA was added as required and the cells equilibrated in the cuvette at 37 °C for several minutes before addition of agonist. The usual cell density was  $1.1 \times 10^8$  ml<sup>-1</sup>. ATP secreted into the medium was measured from the luminescence of luciferin-luciferase, added as 20  $\mu$ l ml<sup>-1</sup> 'Chronolume' reagent, and expressed as per cent of that released by 0.5 U ml<sup>-1</sup> thrombin, typically 2 nmol per  $10^8$  platelets. The quin2 fluorescence was measured in a Perkin Elmer MPF 44A spectrophotometer with excitation at 339 nm and emission at 500 nm essentially as previously described<sup>5</sup>. All measurements were made at 37 °C. OAG, prepared by a modification of the method of Buchnea<sup>35</sup>, was a gift from Dr R. Y. Tsien.



produced by OAG and TPA. If diacylglycerol is the trigger for these responses, it follows that collagen and thrombin can stimulate formation of diacylglycerol while  $[Ca^{2+}]_i$  remains at basal levels. Thus agonist-induced phosphoinositide breakdown does not necessarily require elevated  $[Ca^{2+}]_i$ . This appears to be the case in several systems<sup>24-27</sup>. In platelets, thrombin and collagen cause formation of diacylglycerol in the absence of external calcium and independent of thromboxane formation<sup>9,10,12,23</sup>. These data, together with our finding that collagen does not elevate  $[Ca^{2+}]_i$  in aspirin-treated cells, indicate that diacylglycerol can be formed at basal  $[Ca^{2+}]_i$ . Our own preliminary measurements show that in conditions like those for Fig. 2, collagen and thrombin do stimulate phosphoinositide breakdown via phospholipase C (R. F. Irvine, T.J.H., A.S. and T.J.R., unpublished).

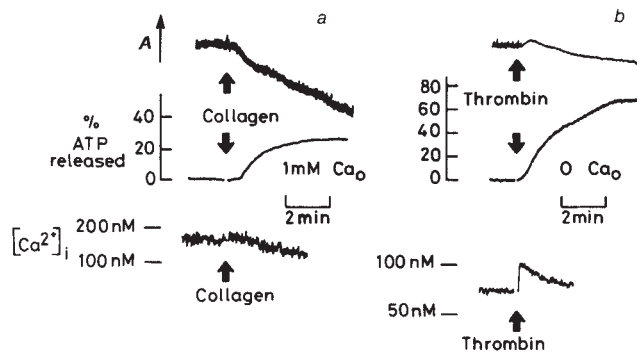
Interestingly, results such as those in Fig. 2 do suggest that a further excitatory intracellular signal, other than calcium or diacylglycerol, is involved in some aspects of platelet activation. The shape-change and discharge of intracellular calcium stores that are stimulated by thrombin, but not collagen, probably cannot be attributed to diacylglycerol as neither OAG nor TPA produced these effects. The putative third message is unlikely to be thromboxane, since the experiments are done in the presence of aspirin, nor cyclic nucleotides which inhibit platelet function. One attractive possibility is inositol triphosphate, an immediate product of the breakdown of phosphatidylinositol biphosphate which has recently been reported as a rapid response to thrombin<sup>28</sup>.

Although it seems that calcium and diacylglycerol can each separately stimulate secretion, there is evidence for potentiation

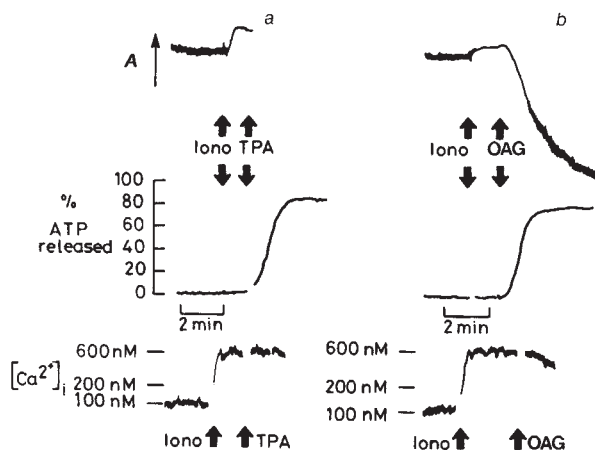
between them (ref. 11 and Fig. 3). When a low concentration of ionomycin was first added to raise  $[Ca^{2+}]_i$  to ~600 nM, which was sub-threshold for secretion, subsequently applied TPA or OAG evoked a much faster secretion (and aggregation) than that seen at basal  $[Ca^{2+}]_i$ . The responses now more closely resembled those normally produced by strong agonists such as thrombin and PAF, which both raise  $[Ca^{2+}]_i$  and promote diacylglycerol formation. The site of interaction between calcium and diacylglycerol could be C-kinase itself. *In vitro* studies indicate that diacylglycerol<sup>11</sup> and TPA<sup>29</sup> can activate the kinase independently of calcium, that 1–50  $\mu$ M calcium can activate the kinase independently of diacylglycerol<sup>30</sup>, and that in the presence of diacylglycerol the apparent affinity for calcium is increased. Unfortunately, effects of  $[Ca^{2+}]_i$  in the range 0.1–1.0  $\mu$ M have not been reported. It is possible that sub-threshold elevation of  $[Ca^{2+}]_i$  may enhance the secretory response to diacylglycerol by means other than increasing the activation of C-kinase<sup>11</sup>. A concentration of calcium ionophore too low to stimulate secretion was reported to potentiate the diacylglycerol-evoked secretion without increasing the phosphorylation of the 40,000-molecular weight substrate<sup>11</sup>. This may imply that some process other than the phosphorylation is rate-limiting and can be accelerated by modest rises in  $[Ca^{2+}]_i$ . Whatever the mechanism, the interaction between calcium and diacylglycerol highlights the possible importance of modulation of  $[Ca^{2+}]_i$  in a range where it may not, by itself, be an effective activator.

The relative importance of diacylglycerol and calcium as triggers for exocytosis will presumably vary in different cell types, and the occurrence of secretory exocytosis need not be indicative of a rise in  $[Ca^{2+}]_i$ . In some tissues, such as juxtaglomerular





**Fig. 2** Collagen and thrombin stimulate secretion at basal  $[Ca^{2+}]_i$ , and without thromboxane formation. Experimental conditions as for Fig. 1. *a*, Responses of aspirin-treated platelets in 1 mM  $Ca$  medium to  $10 \mu g ml^{-1}$  collagen (Hormon-Chemie). *b*, Secretion produced by  $0.5 U ml^{-1}$  thrombin in aspirin-treated cells in nominally  $Ca$ -free medium containing 1 mM EGTA. The rise in  $[Ca^{2+}]_i$ , presumed to be due to release of intracellular  $Ca$ , is small because the cells were loaded with  $\sim 2.5$  mM quin2, about twice that normally used.



**Fig. 3** Sub-threshold rises in  $[Ca^{2+}]_i$  accelerate the secretory responses to TPA and OAG. Experimental conditions as for Fig. 1. The platelets were loaded with  $\sim 1$  mM quin2 and preincubated with 100  $\mu M$  aspirin. The external  $[Ca^{2+}]_o$  was 1 mM. *a*, Response to 10 nM ionomycin followed by 20 nM TPA; *b*, response to 5 nM ionomycin followed by  $60 \mu g ml^{-1}$  OAG.

apparatus<sup>31</sup> and parathyroid gland<sup>32</sup>, the available indirect evidence suggests that elevated  $[Ca^{2+}]_i$  is inhibitory, and very recent direct measurements with quin2 in bovine parathyroid cells confirm this idea<sup>33</sup>. Diacylglycerol is an obvious candidate for the secretory trigger in these cell types. When very rapid transient secretion is required, as with neurotransmitter release, the system may be set up to be activated by just a brief influx of calcium. In other cells, such as platelets and polymorphs<sup>34</sup>, both triggers may be involved in the secretion produced by natural agonists.

It also seems likely that diacylglycerol has actions in addition to initiating secretory exocytosis. In our experiments with diacylglycerol, the disk-to-sphere transition and platelet aggregation observed involve more than secretion of granule contents. The wide range of effects of TPA also suggests that activation of C-kinase by naturally formed diacylglycerol has a variety of functions in many cell types. An immediate product of phosphoinositide breakdown may be about to take a place alongside calcium and the cyclic nucleotides as a widespread, if not universal, cellular regulator.

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## Different antiviral spectra of human macrophage interferon activities

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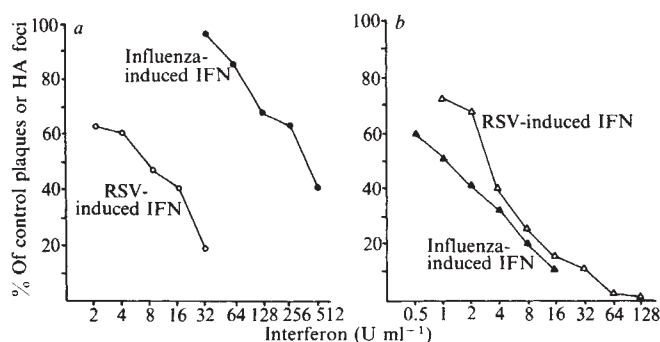
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The antiviral activity of  $\alpha$ -interferon (IFN- $\alpha$ ) produced by human leukocytes in response to viral infection has been considered to be independent of the virus which induced its production. Recently, however, IFN- $\alpha$  has been found to include at least eight different subtypes, as indicated by measurement of antigenic variability, DNA hybridization and amino acid sequencing<sup>1-3</sup>. We considered the possibility that interferon heterogeneity may play a part in enhancing host antiviral defence and now present data suggesting that purified human macrophages, when exposed to different viruses, produce interferons having differing spectra of antiviral activity. These findings may provide a functional correlation for IFN- $\alpha$  heterogeneity.

Purified human macrophages were obtained from the peripheral blood of healthy volunteers by Ficoll-Hypaque sedimentation, followed by adherence to plastic Petri dishes and extensive washing to remove nonadherent cells<sup>4</sup>. Macrophages were then exposed for 1 h to influenza A/AA/Marton/43 H1N1 or respiratory syncytial virus (RSV, Long strain), washed extensively and incubated for 24 h at 37 °C (refs 5, 6). Supernatant culture fluids were collected and assayed for interferon-like activity by measuring inhibition of plaque formation by vesicular stomatitis virus (VSV) in human foreskin fibroblast cultures<sup>5</sup>.

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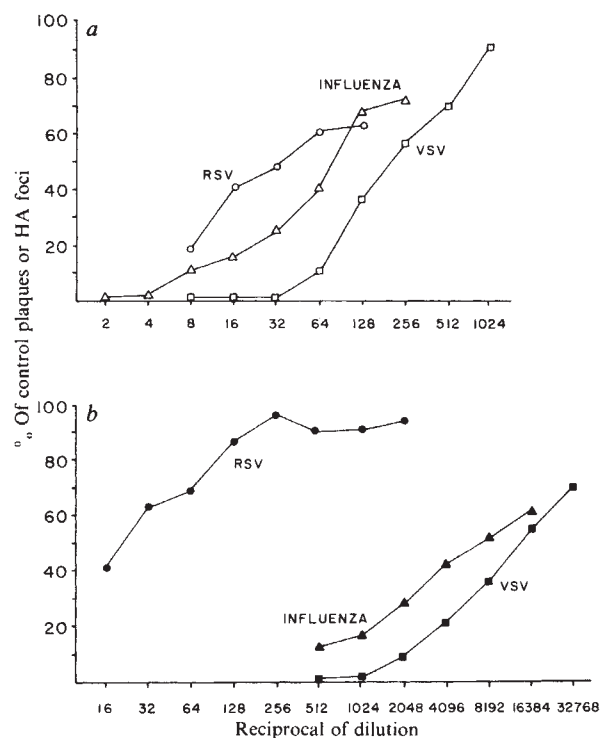
**Fig. 1** Sensitivity of respiratory syncytial virus (RSV) (a) and influenza virus (b) to RSV-induced interferon (IFN) activity and to influenza virus-induced IFN activity. Interferon activity was first defined, in units per ml, by its inhibitory effect on vesicular stomatitis virus (VSV). Subsequently, inhibition of RSV and influenza virus growth in human foreskin fibroblast cultures was quantitated as U ml<sup>-1</sup> required to produce a 50% reduction in viral plaques (RSV) or haemadsorbing (HA) foci (influenza virus). The cultures were exposed to RSV-induced virus (VSV) activity, influenza virus-induced IFN activity or mock IFN activity for 18 h, washed, challenged with either RSV or influenza virus for 3 h, washed again, and then overlaid with medium containing 1% agarose in the plaque assay, or with the medium alone in the haemadsorbing foci assay. Interferons from the cells of three individuals were used, providing equivalent results.

This assay is a commonly used, reproducible method of measuring interferon activity<sup>7</sup>. Each assay was performed in triplicate and incorporated a laboratory interferon standard that contained 3,000 IU ml<sup>-1</sup>, compared with the human research reference standard 69/19. Minimum interferon titres of 5 U ml<sup>-1</sup> were detectable.

The interferon activity produced in response to the viruses was trypsin-sensitive and resistant to pH 2; it showed no direct antiviral properties and was neutralized by antiserum to human IFN- $\alpha$  (refs 5, 8). Thus, the antiviral activity met generally accepted criteria for characterization as IFN- $\alpha$ .

The sensitivities of RSV and influenza virus to the interferon induced by RSV and by influenza virus were then determined. We quantitated virus growth by using a plaque assay for RSV, and by counting haemadsorbing foci in the case of influenza virus<sup>9</sup>. Assays were performed in cultures of human foreskin fibroblasts challenged with virus after pretreatment with the interferon preparations (Fig. 1). Approximately 8 U ml<sup>-1</sup> of RSV-induced interferon produced 50% reduction in plaques in the cultures challenged with RSV. However, ~512 U ml<sup>-1</sup> of influenza virus-induced interferon was required to achieve the same degree of plaque reduction in parallel cultures (Fig. 1a). Thus, RSV appeared to be markedly more sensitive to RSV-induced interferon activity than to influenza virus-induced interferon activity. In contrast, influenza virus showed relatively similar sensitivity to the RSV- and the influenza virus-induced interferons, as approximately 2–4 U ml<sup>-1</sup> of either interferon preparation produced 50% reduction in haemadsorbing foci following influenza virus challenge (Fig. 1b).

These data suggested that differences exist in the spectrum of antiviral activity of interferons produced by purified human macrophages in response to different viruses. However, the activity of a particular interferon preparation was itself defined, in U ml<sup>-1</sup>, by measuring its effect on a third virus, considered to be the reference standard. A differential sensitivity of the reference virus to different interferons could confound our ability to compare activities of these interferons against other viruses. Therefore, we determined directly the relative sensitivities of RSV, influenza virus and the reference virus (VSV) to macrophage-produced interferon activity. Serial two-fold dilutions of RSV-induced or influenza virus-induced human macrophage supernatant fluids were assayed for ability to reduce



**Fig. 2** Sensitivity of respiratory syncytial virus (RSV), influenza virus and vesicular stomatitis virus (VSV) to RSV-induced interferon activity (a) and to influenza virus-induced interferon activity (b), expressed as the reciprocal of the dilution of macrophage supernatant fluids producing a reduction in virus yield. Inhibition of virus growth in human foreskin fibroblast cultures was quantitated as a reduction in viral plaques (RSV, VSV) or haemadsorbing (HA) foci (influenza virus).

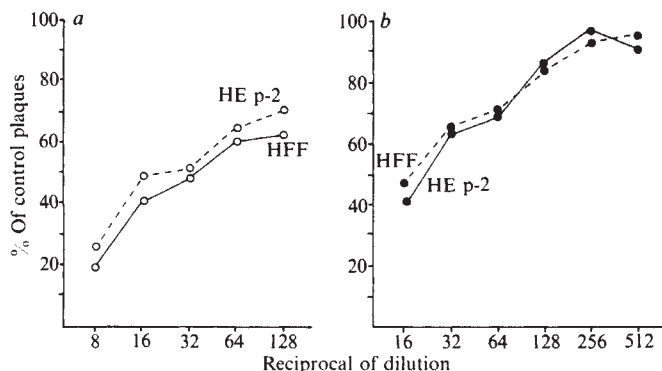
plaques or haemadsorbing foci on challenge by the three viruses. Thus, the sensitivity of VSV would not affect the determinations of relative RSV and influenza virus sensitivities.

As noted earlier<sup>6,10</sup>, RSV induced less interferon activity overall than did influenza virus. The three viruses were sensitive, to an almost equivalent degree, to the antiviral activity of RSV-induced interferon, only one- to threefold differences being noted in dilutions of antiviral activity required to obtain 50% reduction in viral plaques or haemadsorbing foci (Fig. 2a). Influenza virus and VSV were equally susceptible to influenza virus-induced antiviral activity, with similar dilutions of the preparation resulting in 50% reduction in haemadsorbing foci or plaques (Fig. 2b). In contrast, RSV sensitivity to the influenza virus-induced antiviral activity was markedly different, requiring ~256-fold more such interferon to obtain 50% reduction in viral plaques.

The antiviral activity of interferon is indirect, inducing synthesis of antiviral proteins in interferon-treated cells<sup>11</sup>. Thus, the differing relative anti-RSV activities of our macrophage-produced interferons could be due totally or in part to the nature of the cell challenged with virus, rather than be specific to the challenging virus. To determine the site of relative specificity, we repeated the RSV challenge experiments in a different cell line. Results were identical in both human foreskin fibroblasts and HEP-2 cells, treated with either RSV-induced or influenza virus-induced interferon and challenged with RSV (Fig. 3). Furthermore, sensitivities of influenza virus to RSV-induced or influenza virus-induced interferons were similar when assays using HEP-2 cells were compared with those using human foreskin fibroblasts (data not shown).

The data suggest that human macrophages produce interferons having differing spectra of antiviral activity in response to different viruses. Others have shown that the host cell could be involved in determining the activity of interferon against different viruses<sup>12,13</sup>. This has been demonstrated in animal





**Fig. 3** Sensitivity of respiratory syncytial virus (RSV) to RSV-induced interferon activity (a) and to influenza virus-induced interferon activity (b). Inhibition of RSV growth in human foreskin fibroblast (HFF) or HEp-2 cultures was quantitated as a reduction in viral plaques.

studies, by using a single interferon preparation. In the present studies, the relative specificity of antiviral activity of the human macrophage interferons, induced by different viruses, appeared to be related to the viruses themselves rather than to the type of human cell with which sensitivity was assayed. Of course, we cannot yet exclude the possibility that other types of human cells would demonstrate significant variability in antiviral activity after treatment with these interferons. Nonetheless, it seems that the relative specificity of antiviral activities described here cannot readily be ascribed to cell specificity.

The existence of multiple subtypes of IFN- $\alpha$  may be better understood if the subtypes possess different spectra of activity against the many viruses that humans encounter. We cannot exclude the possibility that the observed differences in antiviral activity were due to the presence of other factors, produced concomitantly by the virus-exposed macrophages. The antiviral activities met generally-accepted criteria for characterization as IFN- $\alpha$ , as noted above. However, other substances that might modify IFN- $\alpha$  activity could be present nonetheless.

Our data suggest that standard interferon assays using reference challenge viruses, such as VSV, may not adequately reflect the therapeutic potential of a particular interferon. Variation in relative antiviral spectra of different IFN- $\alpha$  preparations, whether induced by virus exposure of buffy coat leukocytes or produced by recombinant DNA synthesis methods, may partly explain the varied therapeutic efficacies reported by different investigators with treatment of infections due to the same virus<sup>14-17</sup>. Our data raise the possibility that effective use of interferon as an antiviral agent requires analysis of the subtype compositions of interferon induced by different viruses, and determination of the spectra of relative antiviral activity attributable to the different subtypes.

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## Characterization of melanin-concentrating hormone in chum salmon pituitaries

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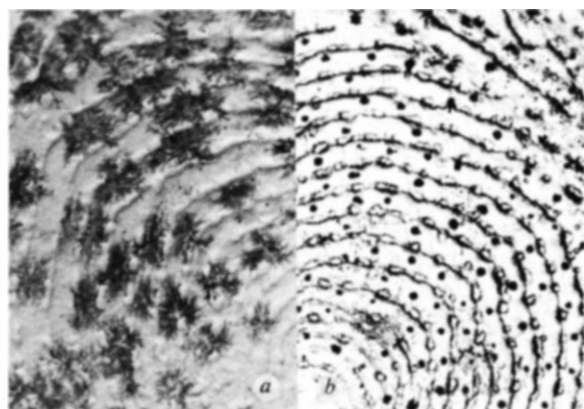
Many lower vertebrates exhibit colour change in response to the background. A dual hormonal control of colour change by two antagonistic pituitary melanophorotropic hormones was first postulated in amphibia by Hogben and Slome<sup>1</sup>. It is well established that the melanotropins  $\alpha$ - and  $\beta$ -MSH are responsible for pigment dispersion in the integumentary melanophore of lower vertebrates and that these molecules are derived from a common precursor protein, proopiomelanocortin, by specific processing within the intermediate lobe<sup>2</sup>. No evidence has been found for an antagonistic hormone in amphibia, although the existence of such a molecule in the pituitary gland of teleost fishes has long been recognized and was termed the melanophore-concentrating hormone by Enami<sup>3</sup>. Early attempts<sup>4,5</sup> to separate the two hormones proved unsuccessful. Recently, Baker and Ball<sup>6</sup> re-invoked the dual hormone concept, and it has been suggested<sup>7</sup> that a melanin-concentrating hormone (MCH) is synthesized in the hypothalamus of teleosts and stored and released by the neurohypophysis. We have now isolated a novel peptide from the pituitary of the salmon (*Oncorhynchus keta*) possessing an antagonistic function to MSH, and we describe here its chemical and biological characteristics.

Our previous studies on salmon proopiomelanocortin-related peptides revealed two molecular forms of  $\alpha$ -MSH,  $\beta$ -MSH and six related peptides in the pituitary extracts<sup>8-11</sup> but no  $\gamma$ -MSH-like peptide<sup>11,12</sup>. Based on the earlier observation mentioned above, we initially suspected that the melanin-concentrating activity might be attributable to one or other of the different  $\alpha$ - or  $\beta$ -MSHs, which are distinguishable by ion-exchange and/or partition chromatography, as well as by electrophoresis. However, the melanin-concentrating assay of Rance and Baker<sup>7</sup> revealed no biological activity for these MSHs, or for Arg-vasotocin<sup>11</sup>, endorphins<sup>13,14</sup>, corticotropin-like intermediate lobe peptides<sup>15,16</sup>, N-terminal peptides of proopiomelanocortin<sup>12</sup>, prolactin<sup>17</sup>, growth hormone or gonadotropins (unpublished data), all of which we have isolated from the teleost pituitary. Thus, it seemed that MCH was an entirely new molecule. Despite previous attempts to isolate and characterize MCH<sup>3,5,18,19</sup>, its structure has remained unknown.

The *in vitro* method of Rance and Baker<sup>7</sup>, with slight modification, was used to identify MCH during fractionation. Scales were taken from yearling tilapia *Sarotherodon mossambicus* instead of from trout, and were incubated at 20 °C, instead of at the lower temperatures used for trout, in a Tyrode Ringer containing 0.1 mM phentolamine. This modified system gave reproducible and consistent results. During a 30-min preincubation period the melanin granules on the scales became fully dispersed to a melanophore index<sup>1</sup> of about 5 (Fig. 1a). Three of these scales were then incubated in the test solution containing the sample dissolved in Tyrode-phentolamine Ringer; after 20 min they were examined under a binocular microscope.

The salmon MCH was extracted from whole pituitary glands with acid acetone. The extract was subjected to gel filtration on

a Sephadex G-25 column in 0.1 M acetic acid according to the protocol adopted for the proopiomelanocortin peptides<sup>8-16</sup> and for prolactin<sup>17</sup>. The activity was found in the retarded fraction III (ref. 9). This fraction was desalted by ultrafiltration using a UM 2 membrane (Amicon) and chromatographed on a CM-cellulose column as shown in Fig. 2. Only the eighth fraction, which eluted just before  $\alpha$ -MSH I, exhibited biological activity. Final purification was achieved by HPLC on a reverse-phase C18 column, eluted with a linear gradient of 0.1% trifluoroacetic acid and acetonitrile (Fig. 3). A final yield of 0.4 mg was obtained from 50 g (wet weight) of the pituitaries.



**Fig. 1** Effect of salmon MCH on melanin granules in tilapia scale melanophores. *a*, Melanin granules on a scale of tilapia (*S. mossambicus*) become fully dispersed in a Tyrode Ringer containing 0.1 mM phentolamine. *b*, Melanin granules become fully concentrated after incubation in Tyrode-phentolamine Ringer containing 50 nM MCH.

Chemical characterization revealed that the hormone was a novel peptide and not a catecholamine. Salmon MCH consists of 17 amino acids with the following composition: Asx 1.0, Thr 1.0, Glx 1.0, Pro 1.2, Gly 1.1, 1/2 Cys 1.9, Val 3.0, Met 2.0, Tyr 0.8, Arg 2.5, Trp 1.2, with a single N-terminal residue, Asp, and a single C-terminal residue, Val. The amino acid sequence of the reduced and carboxymethylated MCH was determined by the fluorescein isothiocyanate method<sup>20</sup>:

H-Asp-Thr-Met-Arg-Cys-Met-Val-Gly-Arg-Val-Tyr-Arg-  
Pro-Cys-Trp-Glu-Val-OH.

To confirm this structure, the MCH (~50 nmol per sample) was digested in 0.2 M ammonium acetate buffer, pH 8.0, for 1 h with chymotrypsin and for 4 h with trypsin at 37 °C. The resulting peptides were fractionated by HPLC on the reverse-phase C18 column with a gradient of 10 mM ammonium acetate, pH 5.0, and isopropanol. The peptides obtained were analysed by N-terminus, amino acid composition and amino acid sequence by the Edman-dansyl method<sup>21</sup>. Chymotryptic peptides:

C-1 (H-Asx-Thr-Met-Arg-OH),  
C-2 (H-Val-Gly-Arg-Val-Tyr-OH)

and

C-3(H-Cys-Met-OH) (H-Arg-Pro-Cys-Trp-Glx-Val-OH);

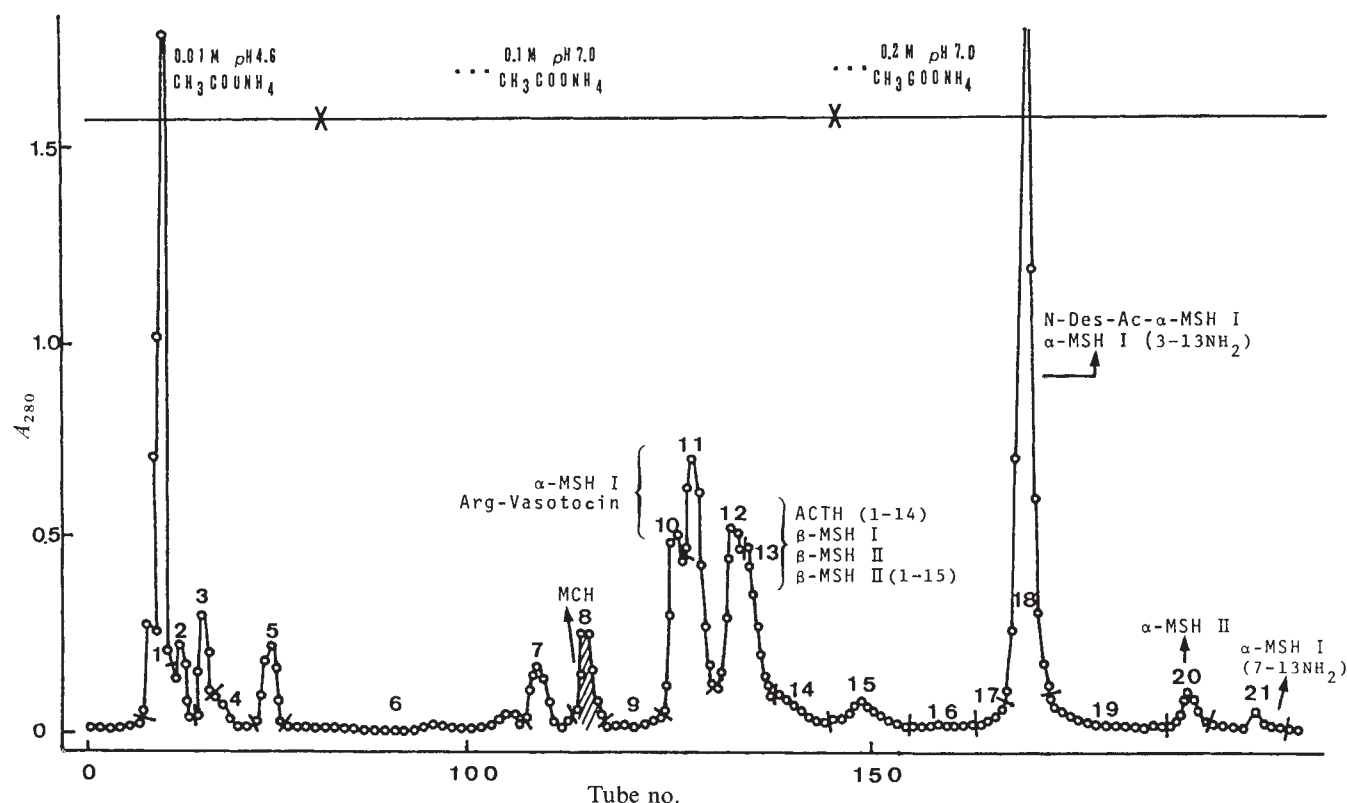
tryptic peptides:

T-1 (H-Asx-Thr-Met-Arg-OH),  
T-2 (H-Val-Tyr-Arg-OH),

and

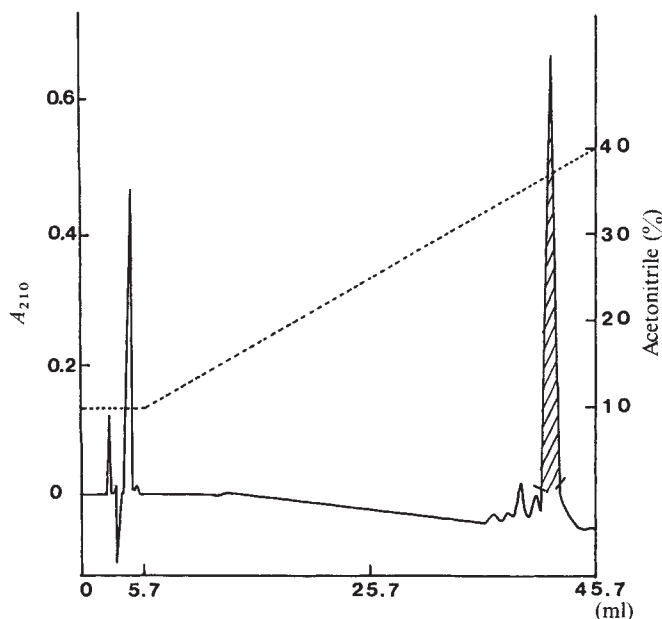
T-3 (H-Cys-Met-Val-Gly-Arg-OH)  
(H-Pro-Cys-Trp-Glx-Val-OH).

A disulphide bond was found in C-3 and T-3. The C-terminal sequence -Glu-Val-OH was also confirmed by kinetic studies



**Fig. 2** Ion-exchange chromatography of fraction III on CM-cellulose column (1.6×63 cm) by gradient elution as shown. Fraction III (75 mg) was obtained from an acid acetone extract (1.3 g) of salmon pituitaries (50 g) by exclusion chromatography of Sephadex G-25, using the procedure described previously<sup>9</sup>. The effluent was monitored by measuring the absorbance at 280 nm and was collected in 10-ml fractions. Flow rate was 60 ml h<sup>-1</sup>. A 0.1 mg sample of each fraction was dissolved in 2 ml of Tyrode-phentolamine solution and tested for melanin-concentrating activity with tilapia scales. Only fraction 8 exhibited significant activity, which was detectable even at 1,000-fold dilution. The yield of the fraction was 0.7 mg. The molar amounts of  $\alpha$ -MSH I,  $\alpha$ -MSH II and MCH obtained from the salmon pituitary extract were 1, 0.8 and 0.5  $\mu$ mol, respectively.





**Fig. 3** HPLC of fraction 8 on a reverse-phase column (Finepack sil C18, 10  $\mu$ m particle size, 4.6 $\times$ 250 nm) by a gradient elution. The material (0.1 mg for each run) was loaded, and the effluent was monitored by measuring the absorbance at 210 nm. Flow rate was 1.6 ml min<sup>-1</sup>.

of carboxypeptidase Y digestion of the intact peptide. These results support the structure proposed above and the disulphide bridge formed between residues 5 and 14.

The salmon MCH was effective at a concentration of 1 nM, which caused the melanin granules in 50% of the melanophores on a tilapia scale to become fully concentrated down to melanophore index 1. This was 10<sup>4</sup>–10<sup>5</sup> times more potent than catecholamines, which are well known melanin-concentrating agents in many teleost species. The salmon MCH proved active at the same low concentration on all teleost scales tested, including trout (*Salmo gairdneri*), carp (*Cyprinus carpio*), black rockfish (*Sebastes schlegeli*) and fat greenling (*Hexagrammos otakii*), which suggests that MCH may be a general teleostean hormone. Comparison of the amino acid sequence of MCH with those of all known brain and pituitary proteins revealed similarity between MCH and the carboxyl-terminal disulphide loop of salmon prolactin<sup>17</sup>, but not of mammalian prolactins. In fact, a chymotryptic peptide of the salmon prolactin

(H-Arg-Cys-Ala-Thr-Lys-Met-Arg-Pro-Glu-Thr-Cys-OH)

exhibited very weak MCH activity (5 $\times$ 10<sup>-4</sup> times less potent than MCH).

The antagonistic interaction between MSH and MCH can also be demonstrated using the *in vitro* scale bioassay. Tilapia scales which showed full melanin dispersion in Tyrode-phenolamine Ringer (Fig. 1a) were incubated in test solutions containing 50 nM MCH (Fig. 1b) and various amounts of salmon  $\alpha$ -MSH I, which is identical in structure to mammalian  $\alpha$ -MSH<sup>8,11</sup>. At an equimolar ratio, all the melanophores showed full melanin concentration within 20 min, followed by gradual melanin dispersion. Even at a higher ratio of  $\alpha$ -MSH I, the effect of MCH appeared first, although the melanin granules did not become fully concentrated (data not shown). This antagonism explains the inability of crude teleost pituitary extracts to induce melanin concentration in certain fish such as the eel *Anguilla*, even though such species are able to respond to partially purified MCH<sup>22</sup>. Presumably the relative sensitivity of melanophores to MCH and MSH varies for different teleost species.

The present investigation thus further substantiates the hypothesis of a dual humoral control of colour change. However, the precise site of origin of MCH remains uncertain. Although melanin-concentrating activity has been found in the pituitary

of many teleost species<sup>3,7,23</sup>, Enami<sup>3</sup> and Rance and Baker<sup>7</sup> suggested that MCH was a neurosecretory product from the hypothalamus, and was stored and released by the neurohypophysis. In recent work Rance and Baker have attempted to map the localization of MCH biological activity within the trout brain<sup>22</sup>. The MCH we identified was present in the pituitary fraction. More accurate localization should soon be possible using specific antiserum against MCH.

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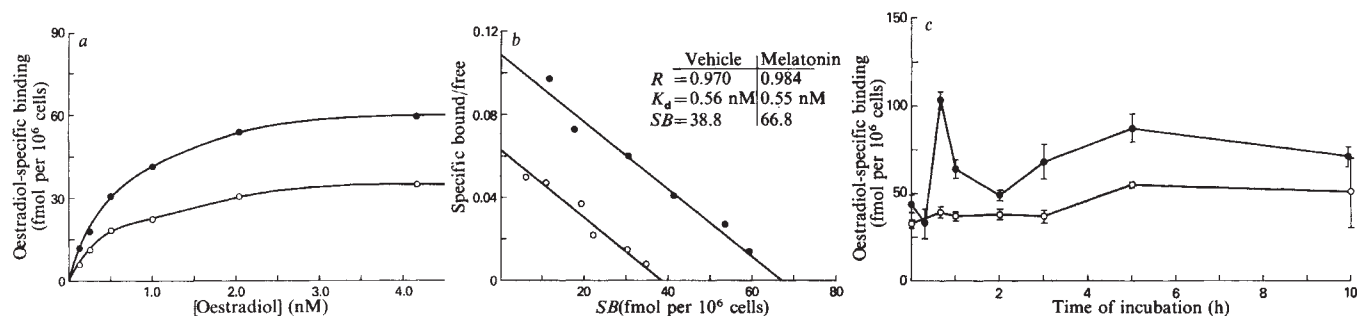
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## Melatonin increases oestrogen receptor binding activity of human breast cancer cells

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Breast cancer is frequently a hormone-dependent tumour, and several studies have suggested that the pineal gland hormone melatonin may influence the growth and development of this malignancy. Subcutaneous injections of melatonin have been shown to inhibit, and pinealectomy to enhance, the development of dimethyl benz(a)anthracene (DMBA)-induced mammary tumours in rats<sup>1</sup>. Use of the psychotropic drug thiorazine, which increases plasma melatonin levels<sup>2</sup>, has been associated with a decreased incidence of breast cancer in psychiatric patients<sup>3</sup>. Calcification of the pineal gland has been correlated with an increased incidence of breast cancer in women<sup>4</sup>. While the mechanism by which melatonin influences these tumours is unknown, both human breast cancer and DMBA-induced tumours contain oestrogen receptors (ER) and respond to changes in the oestrogen milieu<sup>5–7</sup>. We therefore wondered whether melatonin might be altering ER binding activity of these tumours. We report here that *in vitro* incubation of MCF-7 human breast cancer cells with melatonin in physiological conditions increased the cytoplasmic and nuclear ER activity of these cells within 40 min, giving no change in the equilibrium dissociation constant ( $K_d$ ) of the receptor. This induction was blocked by cycloheximide, and thus requires continuous protein synthesis. The modulation of ER binding activity of breast cancer by another endogenous hormone may be important for understanding the behaviour and treatment of this disease, and may provide insight into the factors regulating the synthesis and metabolism of steroid hormone receptors.



**Fig. 1** Effect of melatonin on oestradiol-specific binding of MCF-7 cells. **a**, Oestradiol-specific binding curve of vehicle (○) and melatonin (●)-treated cells. MCF-7 cells were grown to confluence in 6-well Corning plates as described elsewhere<sup>18</sup>. Melatonin (1 nM) was added to the medium for 60 min, and the medium then replaced with Eagle's MEM containing glutamine (0.25 g l<sup>-1</sup>), tricine (0.1 l<sup>-1</sup>) and various concentrations of <sup>3</sup>H-oestradiol with or without 200-fold excess DES and the incubation continued for 30 min. The monolayer was washed three times with PBS, then the cells were collected and resuspended in 1.0 ml PBS, and an aliquot of the suspension counted in a haemocytometer. The remaining cells were disrupted by vortexing, extracted with 1.0 ml ethanol overnight, and the radioactivity counted in 8.0 ml of scintillation fluid. Oestradiol-specific binding was determined by Scatchard analysis<sup>9,10</sup>. These data are representative of five experiments. **b**, Scatchard analysis of equilibrium binding curve in **a**.  $R$ , regression coefficient;  $K_d$ , equilibrium dissociation constant;  $SB$ , specific binding (fmol per 10<sup>6</sup> cells). **c**, Time course of melatonin induction of ER activity. Cells were incubated with 1 nM melatonin for the time periods shown. Oestradiol-specific binding was measured using a saturating concentration (5 nM) of <sup>3</sup>H-oestradiol with or without 200-fold excess DES by the whole cell assay method described for **a**. Each time point represents the mean  $\pm$  s.e.m. for 2–3 experiments. ○, Vehicle alone; ●, melatonin.

We first studied the effect of melatonin on the total ER binding activity of MCF-7 human breast cancer cells. These cells are derived from the malignant pleural effusion of a patient with hormone-dependent breast cancer. We chose for our initial studies a melatonin concentration of 1 nM, which approximates the physiological peak night-time plasma level in humans<sup>8</sup>. When MCF-7 cells were incubated with melatonin (1 nM) for 60 min, a 78% increase ( $P < 0.01$  for 5 experiments) in ER binding activity occurred (Fig. 1a). Scatchard analysis<sup>9,10</sup> of this binding curve revealed that the induced receptor population was homogenous, with an equilibrium dissociation constant ( $K_d$ ) comparable to that of control cells (Fig. 1b). Melatonin thus acts directly on human breast cancer cells to increase their ER binding activity. This increased activity is not accompanied by an alteration in the affinity of the receptor for oestradiol.

The induction of ER binding activity by melatonin was dose-dependent: melatonin at a concentration of 10 nM also increased ER activity by 68% ( $P < 0.05$ ), while at concentrations of  $\leq 0.10$  nM it had no effect. Using a melatonin concentration of 1 nM we then studied the time course of the melatonin induction. Enhancement of ER binding activity occurred within 40 min after exposure to melatonin, and increased activity persisted for at least 5 h in the presence of melatonin (Fig. 1c). We have also performed comparable studies of the effect of melatonin on immature hamster uteri<sup>11</sup>, and found that a single subcutaneous injection of melatonin increased uterine ER activity within

20 min; this effect, however, returned to control levels by 2 h after injection. These differences in the duration of induction *in vitro* and *in vivo* are probably explained by differences in the fate of melatonin in the two conditions. MCF-7 cells do not metabolize melatonin; the concentration of added melatonin in the medium remained constant for at least 10 h of incubation (unpublished observations). Melatonin *in vivo*, on the other hand, is metabolized in the liver and excreted in the urine, with a plasma half life of 15 min<sup>12</sup>. The induction of ER activity by melatonin seems to be reversible, and is prolonged by the continued presence of melatonin.

These findings represent an important example of one hormone acting to rapidly increase the number of specific receptor binding sites for another hormone. There are, it would seem, at least three possible mechanisms to explain this action: (1) Melatonin could stimulate net accumulation of ER by altering synthesis or degradation. (2) It could act directly on the ER to induce a conformational change, activating otherwise concealed sites. (3) It could promote the synthesis of an intermediate factor which then induces activation of ER binding activity. Because of the rapid nature of the induction we hypothesized that melatonin may be acting on protein synthesis. To test this, we used cycloheximide, which completely blocks protein synthesis within 20 min in MCF-7 cells<sup>13</sup>. We preincubated the cells with cycloheximide for 60 min, then added melatonin (1 nM) to the medium for 40 min. In these conditions, cycloheximide completely blocked the induction of ER activity by melatonin (Table 1). This induction therefore requires continuous protein synthesis, although it is evident within 40 min. Our data do not allow us to distinguish between enhanced receptor synthesis and synthesis of an intermediary protein which alters receptor conformation. The observation by others that the ER in MCF-7 cells have a half life of 2.5 h suggests that the synthesis of this protein may be rapid<sup>14</sup>; our finding that ER binding of control cells remained unchanged through 1.7 h of incubation in the presence of cycloheximide (Table 1) also suggests that protein synthesis may be involved in the metabolism of ER.

Previous work has suggested that unoccupied ER of MCF-7 cells exist in both the cytoplasm ( $R_c$ ) and nucleus ( $R_n$ )<sup>15</sup>. Whether these represent two different forms of the receptor, or the same form distributed artifactually by homogenization and nuclear washing, is unclear<sup>16</sup>. We thought it important, therefore, to qualitatively and quantitatively evaluate both  $R_c$  and  $R_n$  with respect to melatonin treatment, as well as to determine whether the melatonin-induced ER activity seen in the whole cell is represented by corresponding increases in one or both of these cellular components. We found that exposure of the cells to melatonin (1 nM for 40 min) increased the binding

**Table 1** Effect of cycloheximide on melatonin induction of ER binding activity

| Study group               | Oestradiol-specific binding (fmol per 10 <sup>6</sup> cells) |
|---------------------------|--------------------------------------------------------------|
| Vehicle                   | 37.2 $\pm$ 4.5                                               |
| Vehicle + cycloheximide   | 39.2 $\pm$ 2.8                                               |
| Melatonin                 | 53.6 $\pm$ 3.5*                                              |
| Melatonin + cycloheximide | 39.6 $\pm$ 3.9                                               |

MCF-7 cells (given by Dr M. Rich, Michigan Cancer Foundation, Ann Arbor) were grown to confluence in T-150 Corning flasks as described elsewhere<sup>18</sup>. The monolayer of cells was incubated with vehicle or 20  $\mu$ M cycloheximide for 60 min, followed by the addition of vehicle or 1 nM melatonin for 40 min. The monolayers were then washed with phosphate-buffered saline (PBS), collected, and resuspended in Eagle's minimal essential medium (MEM) containing glutamine (0.25 g l<sup>-1</sup>), tricine (0.1 g l<sup>-1</sup>) and various concentrations of <sup>3</sup>H-oestradiol with or without 200-fold excess diethylstilboesterol (DES). The oestradiol-specific binding of the cells was measured by a whole cell assay method<sup>19</sup> and Scatchard analysis<sup>9,10</sup>.

\*  $P < 0.025$  compared with vehicle alone.



**Table 2** Effect of melatonin on cytosolic and nuclear ER binding activity of MCF-7 cells

|                   | Oestradiol-specific binding |                                 |
|-------------------|-----------------------------|---------------------------------|
|                   | fmol per mg DNA             | fmol per 10 <sup>6</sup> cells* |
| Cytosolic binding |                             |                                 |
| Vehicle           | 138.7 ± 22.0                | (2.78 ± 0.4)                    |
| Melatonin†        | 281.8 ± 57.1                | (5.64 ± 1.0)                    |
| Nuclear binding   |                             |                                 |
| Vehicle           | 885.5 ± 66.2                | (17.70 ± 1.3)                   |
| Melatonin‡        | 1,430 ± 110.4               | (28.60 ± 2.2)                   |

A monolayer of cells was incubated with vehicle or melatonin (1 nM) for 40 min, then the cells were resuspended in phosphate buffer (PB: 5 mM dibasic sodium phosphate, 1 mM monothioglycerol, 10% (v/v) glycerol pH 7.4, 4 °C), homogenized in a Dounce homogenizer with 35 strokes, and the nuclei separated at 800g, washed, and resuspended in sucrose phosphate buffer (SPB: 5 mM dibasic sodium phosphate, 1 mM monothioglycerol, 10% (v/v) glycerol, 0.25 M sucrose). The supernatant from the original separation was recentrifuged at 100,000g and the supernatant (cytosol) collected. 100 µl of cytosol (1–2 mg ml<sup>-1</sup> protein) were incubated with 100 µl of <sup>3</sup>H-oestradiol at various concentrations (0.05–2 nM) with or without 200-fold excess DES at 4 °C for 18 h. One ml of a 1:20 dilution of dextran-coated charcoal (2.5% activated charcoal, 0.25% dextran, 0.01 M Tris base) was then added for 20 min with frequent vortexing, the sample centrifuged at 800g for 10 min, and 0.8 ml of the supernatant counted in 8.0 ml of scintillation fluid. Nuclear oestrogen receptor analysis was performed by incubating 350 µl of nuclear suspension (2–3 × 10<sup>6</sup> nuclei ml<sup>-1</sup>) with 350 µl of SPB containing <sup>3</sup>H-oestradiol at various concentrations (0.05–2.0 nM) with or without 200-fold DES at 4 °C for 18 h. Nuclei were then separated at 800g for 15 min, washed three times with SPB, and the pellet extracted in 1.0 ml ethanol at room temperature overnight. The extract was counted in 8 ml of liquid scintillation fluid. Oestradiol-specific binding of both the cytosol and nuclear receptor was calculated by Scatchard analysis<sup>9,10</sup>. The discrepancy between the total cellular receptor as determined by whole cell assay (37.2 fmol per 10<sup>6</sup> cells; Table 1) and compartmental analysis (20.5 fmol per 10<sup>6</sup> cells; this table) is considered secondary to the nature of the respective preparation techniques.

\*MCF-7 cells in our laboratory contain ~20 pg DNA per cell.

†  $P < 0.025$  and ‡  $P < 0.005$  compared with vehicle alone.

activity of both  $R_c$  (by 103%,  $P < 0.025$ ) and  $R_n$  (by 61%,  $P < 0.005$ ; Table 2). The induction of each occurred without a change in the affinity constant ( $K_d$ : for vehicle alone = 0.17 nM, for melatonin = 0.45 nM). Thus, Both  $R_c$  and  $R_n$  are characterized by a comparable sensitivity to melatonin treatment. Whether the biological activity of the receptor(s) is also altered by melatonin is an important question which we are now investigating.

Our finding that the binding activity of a major steroid hormone receptor may be enhanced by another hormone in physiological conditions may provide further insight into the factors regulating the behaviour and metabolism of these receptor proteins. The enhancement of ER activity by melatonin in breast cancer cells may also have important therapeutic and biochemical implications. Because patients with hormone-dependent (ER-positive) breast cancer have a significantly higher response rate to hormonal manipulation<sup>5</sup>, and a longer disease-free survival<sup>17</sup>, factors such as melatonin which increase the cellular ER activity may influence the treatment and course of this disease. Further studies should clarify the importance of this response to melatonin.

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## Modulation of stress-induced ACTH release by corticotropin-releasing factor, catecholamines and vasopressin

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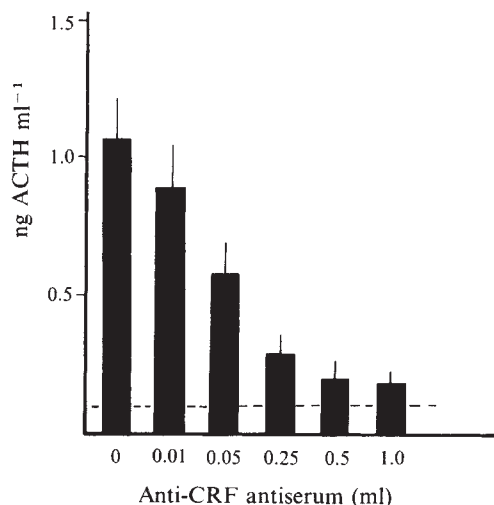
The stress-induced release of ACTH is believed to involve the activation of several humoral and neural pathways, including corticotropin-releasing factor (CRF)<sup>1–4</sup>, catecholamines<sup>5–12</sup> and vasopressin<sup>13–17</sup>. The essential role of CRF was supported by our observation that immunoneutralization of this releasing factor significantly lowers plasma ACTH levels of ether-stressed rats<sup>2</sup>. However, the presence of a small but measurable residual ACTH secretion suggested the possible involvement of factors other than CRF in the stress response<sup>2</sup>. We report here that pretreatment with a vasopressin antagonist decreases the plasma ACTH levels of ether-stressed rats in later (10–20 min), but not earlier (0–10 min), phases of ether stress. The ganglionic blocker chlorisondamine, inhibits ACTH release during both phases of the response to ether by 40–60% when used alone, and by 100% when administered with anti-CRF antibody. These results support a role of CRF, catecholamines and vasopressin in mediating ACTH release by ether stress.

On examining the interaction between various amounts of anti-CRF antibody and stress (Fig. 1) we observed that anti-CRF antibody inhibited ACTH secretion in a dose-related fashion, with a maximum (~85%) blockade being observed after administration of 0.5–1.0 ml of antiserum. These data, which were obtained with an antiserum raised against ovine CRF<sup>1</sup>, are comparable to results observed more recently with an antiserum<sup>18</sup> directed against rat CRF<sup>19</sup>, which indicates that the small residual secretion of ACTH in immunoneutralized ether-stressed rats was probably not due to the inability of the ovine antiserum to totally neutralize rat CRF. This observation suggests that factors other than CRF mediate stress-induced ACTH release.

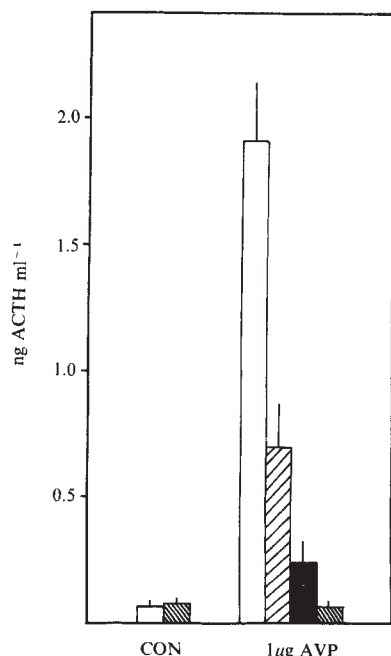
The involvement of various pathways in the activation of the hypothalamic-pituitary axis during stress is well established. While CRF<sup>1–4</sup>, catecholamines<sup>5–12</sup> and vasopressin<sup>13–17</sup> have all been considered as putative mediators of the increased ACTH secretion observed in such circumstances, the evaluation of their respective roles in inducing ACTH release has proven difficult to assess, as apart from their individual ability to act at the pituitary and/or hypothalamic level<sup>20–27</sup>, they can also potentiate each other<sup>20,28–32</sup>. We therefore used specific catecholamine or vasopressin blockers to examine a possible role of catecholamines, which are also secreted during stress<sup>33–40</sup> and are specifically released by central administration of CRF<sup>41,42</sup> as well as of vasopressin, in the ACTH response following ether exposure.

The role of vasopressin in mediating ether stress-induced ACTH release appears to be complex. Vasopressin has been reported to be necessary for the release of ACTH mediated by the paraventricular nucleus of the hypothalamus<sup>43</sup>, which suggests that this peptide might be involved in CRF secretion in certain circumstances. We have observed that the vasopressin

antagonist [1-deaminopenicillamine, 2(*O*-methyl)tyrosine-(dPTyr(Me)AVP)arginine-vasopressin which exhibits both antipressor<sup>44</sup> and anti-ACTH releasing activity<sup>45,46</sup> (Fig. 2), but which does not interfere with CRF-induced ACTH secretion<sup>31</sup>, lowered the ACTH release measured during the late phase of the stress response (that is, at 20 min), but had little effect during the earlier phase (that is, at 10 min) (Fig. 3). The role of vasopressin at the 20-min time point was further emphasized by the ability of the vasopressin antagonist to act in conjunction



**Fig. 1** Effect of various concentrations of anti-CRF antiserum on ether stress induced ACTH secretion. Each point represents the mean  $\pm$  s.e.m. of eight rats. --- Indicates plasma ACTH levels of control animals. Male Sprague-Dawley rats (230–250 g) were equipped with in-dwelling venous catheters<sup>54</sup>. Anti-CRF serum<sup>2</sup> was administered intravenously (i.v.) just before the stress, which consisted of a 3-min exposure to ether (carried out in a room separate from that used for blood sampling). Blood samples were obtained 10 min after the onset of stress.

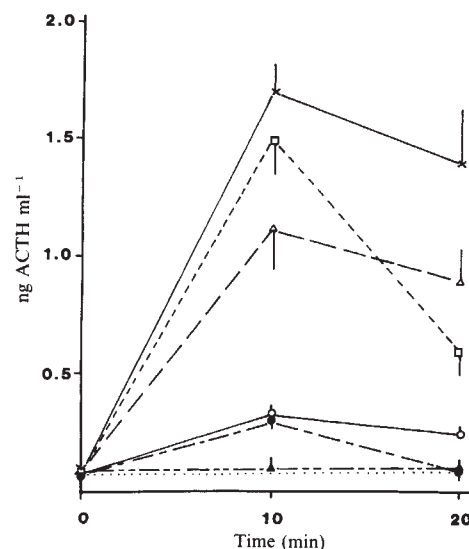


**Fig. 2** Effect of various concentrations of a vasopressin antagonist on AVP-induced ACTH release. This antagonist, dPTyr(Me)AVP, has been shown to reverse the pressor<sup>44</sup>, as well as the ACTH stimulatory activity<sup>45,46</sup> of vasopressin. It was produced by solid-phase synthesis<sup>55</sup>. The rats received saline control (CON), or 1  $\mu$ g AVP 1 min after i.v. administration of 0.05 (▨), 0.11 (■) or 0.25 (▤) mg vasopressin antagonist per kg body weight. Plasma ACTH levels were measured 10 min after injection of AVP. Each point represents the mean  $\pm$  s.e.m. for six rats.

with anti-CRF antiserum to abolish the stress-induced ACTH release at that time (Fig. 3).

Our findings are in contrast with Mormede's report<sup>46</sup> that dPTyr(Me)AVP did not inhibit the increase in ACTH and corticosterone levels due to exposure to a novel environment. It should therefore be emphasized that we used ether stress as a model, and that regulation of ACTH release induced by different types of stressors is known to differ<sup>47</sup>. It is possible that the pure 'psychological' stress used by Mormede<sup>46</sup> did not involve the release of vasopressin, while exposure to ether stress and the possible hypoxia that it may have caused, did increase vasopressin secretion. Indeed, one possible explanation for the delayed ability of the vasopressin antagonist to interfere with the stress response could be that the ether-exposed rats might have developed changes in blood pressure or  $P_{CO_2}$ , which can be associated with increased production of the neurohypophysial peptide<sup>48</sup>.

Having thus established the ability of a vasopressin antagonist to interfere with ACTH secretion due to ether stress, we then investigated a possible role of catecholamines alone, as well as in conjunction with CRF, in mediating stress-induced ACTH release. Catecholamines have long been implicated in regulating ACTH release, but the levels at which they act remain unclear. While catecholamines have been reported to act centrally to both induce<sup>49</sup> and inhibit<sup>11,50,51</sup> the release of CRF, their stimulatory effect at the level of the pituitary is well established<sup>21,22,24–26</sup>. Pretreatment with chlorisondamine, a ganglionic blocker which has minimal access to the brain<sup>52</sup> and acts peripherally to decrease catecholamine release due to stress<sup>36</sup>, typically reduced the stress-induced ACTH release by 40–60% at both the 10- and 20-min time points, while anti-CRF blocked ACTH secretion by 75–85%. This observation suggests that in the stressed rat, the net effect of catecholamines on ACTH secretion is a stimulatory one. Finally, we observed that simultaneous treatment of the animals with chlorisondamine and anti-CRF antiserum consistently resulted in a complete inhibition of the stress response (see Fig. 3). As catecholamines can increase ACTH output by cultured pituitary cells at nanomolar concentrations<sup>21–26</sup>, which fall within the range of



**Fig. 3** Individual and combined effects of a vasopressin antagonist, the ganglionic blocker chlorisondamine<sup>36</sup>, and anti-CRF antiserum on stress-induced ACTH secretion. Groups of six rats were first injected with saline (x), 0.25 mg per kg vasopressin antagonist (□), 3 mg per kg chlorisondamine (Δ), 0.5 ml anti-CRF antiserum (○), or a combination of anti-CRF antiserum and vasopressin antagonist (●), or anti-CRF antiserum and chlorisondamine (▲), and were then submitted to 3 min of ether stress. The rats were decapitated 10 or 20 min after the onset of stress. ... Control (non-stressed) rats. Each point represents the mean  $\pm$  s.e.m. for eight animals.



those found in peripheral blood during stress<sup>53</sup>, it could be expected, as we found, that blockade of both CRF and catecholamine pathways is necessary to completely abolish activation of the hypothalamic-pituitary axis.

The data presented here support the existence of a plurality of factors in modulating ACTH secretion due to ether stress. The observation that the individual administration of a ganglionic blocker, an anti-CRF antiserum or a vasopressin antagonist did not totally block the ether stress response strongly suggests that several neural and humoral pathways are involved in the activation of the hypothalamic-pituitary axis. Furthermore, the changes observed over the time period studied between the interaction of the three inhibitors used, emphasize the complexity of the mechanisms involved in this activation.

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## Inhibition of acetylcholine release from preganglionic frog nerves by ATP but not adenosine

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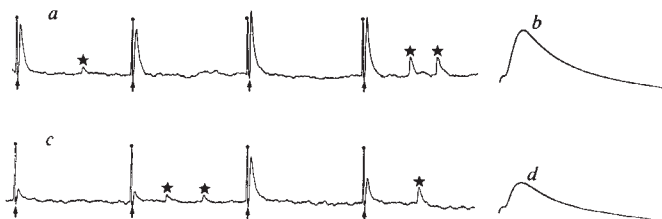
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ATP is known to be released in association with acetylcholine at synapses in the vertebrate peripheral nervous system<sup>1-4</sup>. Exogenously applied ATP and its derivatives have been shown to reduce the release of acetylcholine<sup>5,6</sup>, so it has been postulated that ATP has a role in the modulation of transmitter secretion<sup>2,7</sup>. More recent results have suggested, however, that specific adenosine receptors are responsible for the inhibitory effects of adenosine derivatives on transmitter release, and ATP, if released, must be hydrolysed to adenosine to produce inhibition<sup>8-11</sup>. The original hypothesis that ATP itself might inhibit acetylcholine secretion would be strengthened if it were found that adenosine is very much less potent than ATP as an inhibitor of ACh secretion. We report here results which show this is the case in sympathetic ganglia.

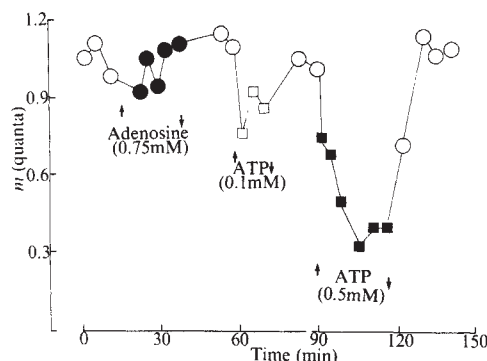
Figure 1 illustrates the effect of ATP on synaptic responses recorded from a ganglion cell in the ninth lumbar sympathetic chain ganglion of the frog (*Rana pipiens*) with an intracellular electrode (for details, see ref. 12 and Fig. 1 legend). The preparation was superfused with Ringer's solution containing 1 mM Ca<sup>2+</sup> and 18 mM Mg<sup>2+</sup> to suppress action potentials. Figure 1a, b shows the synaptic potentials in control conditions; Fig. 1c, d illustrates responses after the addition of 0.5 mM ATP together with sufficient Ca<sup>2+</sup> to compensate for the chelating effect of Ca<sup>2+</sup> on ATP. b and d are the computer-averaged synaptic potentials in response to 88 stimuli (arrows in a and b show the individual evoked synaptic potentials). In this experiment, the mean computer-averaged response was reduced from 21 mV (b) to 12 mV (d). As the miniature synaptic potentials (asterisks in a and c, mean amplitude ~4 mV) were not altered in amplitude by ATP, changes in evoked responses reflect changes in the mean number of acetylcholine quanta released by a nerve impulse ( $\bar{m}$ ). In the experiment of Fig. 1,  $\bar{m}$ , estimated from the fluctuation of the amplitude of the evoked response<sup>13,14</sup>, was reduced from 6.8-3.4 in ATP-containing solution. Similar levels of inhibition (~50% of control) were observed in six other experiments with 0.5 mM ATP. Significant but sometimes smaller inhibitory effects were observed in all four experiments done with lower ATP concentrations (50-250  $\mu$ M, for example Fig. 2).

In view of the frequent suggestion<sup>8-11</sup> that the inhibitory effects of ATP are actually produced by its hydrolysis product, adenosine, the effects of ATP and adenosine on acetylcholine release from preganglionic nerve endings were compared. Figure 2 illustrates an experiment in which transmitter release was estimated by the method of failures<sup>15</sup>. Clearly, in a cell in which 0.1 mM ATP and 0.5 mM ATP were effective in reducing  $\bar{m}$ , 0.75 mM adenosine was without effect. In three other experiments with 0.4-0.75 mM adenosine and in one experiment with 1 mM adenosine, no inhibitory effect on  $\bar{m}$  was observed. In the remaining experiment, 1 mM adenosine reduced  $\bar{m}$  from 2.9 to 2.1. In the presence of 1 mM adenosine, ATP (200  $\mu$ M) continued to inhibit the release of acetylcholine in its characteristic manner. Another adenosine receptor agonist, 2-chloroadenosine, which produces pronounced inhibitory effects at the frog neuromuscular junction in concentrations considerably less than 1  $\mu$ M (refs 9, 15), failed to produce significant changes in  $\bar{m}$  in the present experiments in concentrations ranging from 10 to 100  $\mu$ M (four experiments).

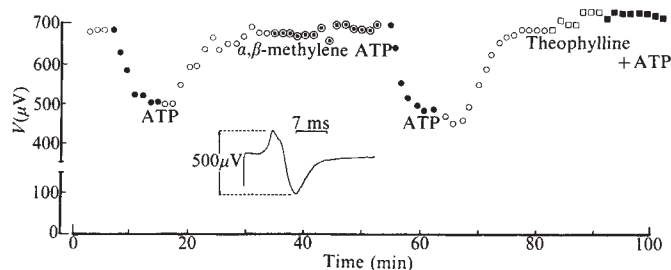
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**Fig. 1** Effect of ATP on synaptic responses recorded from sympathetic chain ganglia in the frog (*R. pipiens*). *a, b*, Responses in control Ringer's solution (1 mM  $\text{Ca}^{2+}$ , 18 mM  $\text{Mg}^{2+}$ , 115 mM NaCl, 2 mM KCl, 2 mM  $\text{NaHCO}_3$ , pH 7.2); *c, d*, responses in control solution containing 0.5 mM ATP (disodium salt; Sigma) plus sufficient calcium to compensate for the chelating effect of ATP. *a*, 4 of the 160 evoked responses (arrows) and 3 of the 70 spontaneous miniature synaptic potentials (\*) in control solution; *b*, 4 of the 180 evoked responses and 3 of the 75 spontaneous miniature synaptic potentials in the ATP-containing solution. The dotted deflections that precede the evoked responses in *a* and *c* are calibration bars of 22 mV; time between arrows, 1 s. *b*, Averaged evoked response to 88 preganglionic nerve stimuli in control solution (21 mV); *d*, averaged response to 88 stimuli in ATP-containing solution (12 mV). The time from the beginning to the peak of the synaptic potentials in *b* and *d* was 10 ms. The nerve was stimulated at a frequency of 1 Hz. In all experiments electrode resistances ranged from 30 to 100 M $\Omega$ . Temperature, 18  $^{\circ}\text{C}$ .



**Fig. 2** Comparison of the effect of adenosine (0.75 mM, ●), ATP (0.1 mM, □) and ATP (0.5 mM, ■) on transmitter release ( $\bar{m}$ ). Each point represents  $\bar{m}$  calculated by the method of failures<sup>13</sup> in response to 128 preganglionic nerve stimuli delivered at a frequency of 1 Hz. ○, Control conditions. For the composition of the control Ringer's, see Fig. 1 legend. Note that ATP but not adenosine depressed  $\bar{m}$  in this experiment.



**Fig. 3** Effects of ATP,  $\alpha,\beta$ -methylene ATP and theophylline on the amplitude of the postganglionic compound action potential. The amplitude of the compound action potential (see inset) reflects the level of synaptic activity in the ganglia (for justification see ref. 17). The *Rana temporaria* preparation was stimulated at 1 Hz in a solution containing 2 mM  $\text{Ca}^{2+}$  and 17 mM  $\text{Mg}^{2+}$ . ○, Control; ●, ATP (200  $\mu\text{M}$ ); ●,  $\alpha,\beta$ -methylene ATP (200  $\mu\text{M}$ ); □, theophylline (2 mM); ■, theophylline (2 mM) + ATP (200  $\mu\text{M}$ ). Note the absence of effect of  $\alpha,\beta$ -methylene ATP on synaptic transmission (a result confirmed with intracellular microelectrode recording) and the antagonism by theophylline of the action of ATP.

In several experiments,  $\alpha,\beta$ -methylene ATP (0.2–0.5 mM), a slowly degradable derivative of ATP, was tested and found to have no effect on acetylcholine release (Fig. 3). In addition, theophylline (2 mM) antagonized the effect of 200  $\mu\text{M}$  ATP (Fig. 3). It thus appears that the receptors responsible for the inhibitory effects of ATP do not resemble  $\text{P}_2$  purinergic receptors<sup>11</sup> or adenosine receptors.

It was reported at a recent symposium<sup>16</sup> that ATP produces a small depolarization of bullfrog ganglia and depresses the output of transmitter from bullfrog preganglionic nerves to a greater extent than adenosine. The results demonstrate gross differences in the sensitivity to purines of different cholinergic nerves, even in the same species. It is likely that these differences extend beyond the structural characteristics of the receptors as spontaneous quantal acetylcholine release from preganglionic nerves (as indicated by the miniature synaptic potential frequency) was unaffected by 0.5 mM ATP (two experiments) while ATP and adenosine produce a parallel depression of  $\bar{m}$  and spontaneous acetylcholine release at the neuromuscular junction<sup>5,6</sup>.

It seems that if ATP is released from active preganglionic nerves, it could reach sufficiently high concentrations at the nerve endings to inhibit the quantal secretion of acetylcholine. The present results, however, make it highly unlikely that adenosine is the purinergic modulator of acetylcholine release in sympathetic chain ganglia.

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## A novel active pentapeptide from chicken brain identified by antibodies to FMRFamide

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The tetrapeptide Phe-Met-Arg-Phe-NH<sub>2</sub> (FMRFamide) and peptides structurally related to it, have been isolated from molluscan ganglia<sup>1–3</sup>. They have widespread actions on both invertebrate and vertebrate tissues and there is increasing evidence that they are an important group of invertebrate peptide neurotransmitters<sup>3–8</sup>. It is of interest that the primary amino acid sequence of FMRFamide forms the C-terminal tetrapeptide of an enkephalin-like heptapeptide (Met-enkephalin-Arg<sup>6</sup>Phe<sup>7</sup>) isolated from bovine adrenal medulla and striatum<sup>9,10</sup>. Antisera to FMRFamide have been shown to

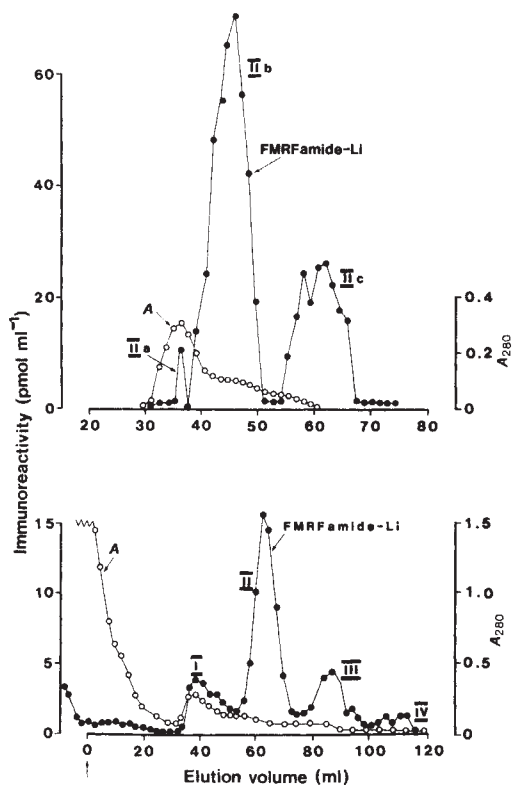


react in radioimmunoassay and immunohistochemistry with material in the central nervous system of various vertebrate species<sup>11-17</sup>, but the identity of this material, and in particular its relationship to the opioid heptapeptide, remains uncertain. We have used antibodies specific for the C-terminus of FMRFamide in radioimmunoassays to monitor purification of the material in chicken brain. We describe here the sequence of one of the peptides obtained. It is a biologically active peptide which does not seem to be related to other known vertebrate neuropeptides.

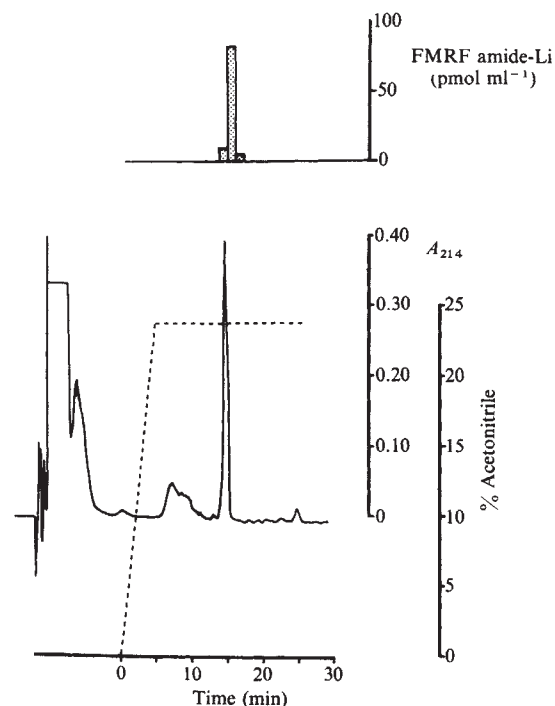
Initial studies of a range of gut and brain tissues from various vertebrates revealed amounts of activity in chicken brain that might be sufficient for isolation. Chicken brains were extracted by boiling in water, followed by acidification with acetic acid to

0.5 M. The extracts were centrifuged and supernatants concentrated by adsorption to C18 Sep-pak cartridges (Waters Associates), elution with acetonitrile and lyophilization. The product was separated into four peaks of immunoreactivity on CM Sephadex ion exchange chromatography (identified as I-IV in order of appearance). The principal peak (II), which correspond to ~50% of total immunoreactivity, was desalted using Sep-pak as before and fractionated by gel filtration on Sephadex G-25 (Fig. 1). At this stage two major peaks (IIb and IIc, which accounted for ~60% and 30-35% of activity, respectively) and one minor peak (IIa) were obtained. Subsequent HPLC studies indicated that peak IIb contained two components, whereas IIc eluted as a single peak of immunoreactivity on three HPLC systems. Thus, after HPLC on C18 and phenyl cartridges in a Z-module (Waters Associates) using an Altex HPLC system, and finally on a C18 Vydac column, a single peak of homogeneous material was recovered (Fig. 2). Amino acid analysis indicated the composition Phe (1), Arg (1), Leu (2) and Pro (1). As the antiserum used for assays shows high specificity for the C-terminal sequence -Arg-Phe-NH<sub>2</sub>, it seemed likely that the chicken brain material would terminate in this sequence. Confirmation was obtained by incubation with trypsin (TPCK trypsin, Worthington; 4 µg, 37 °C, in 40 µl phosphate buffer 0.1 M, pH 7.4), which liberated Phe-NH<sub>2</sub>; this was identified as a single peak of absorbance at 212 nm (retention time 14.8 min) after isocratic HPLC using 3% acetonitrile in 0.1% trifluoroacetic acid on a C18 Vydac column (5×250 mm). This system separates free Phe (retention time 20.5 min), Phe-NH<sub>2</sub> (14.8 min), Tyr (11.0 min) and Tyr-NH<sub>2</sub> (7.6 min). Standardization of the system with graded amounts of Phe-NH<sub>2</sub> (44-360 pmol) indicated that the limit of detection was about 50 pmol.

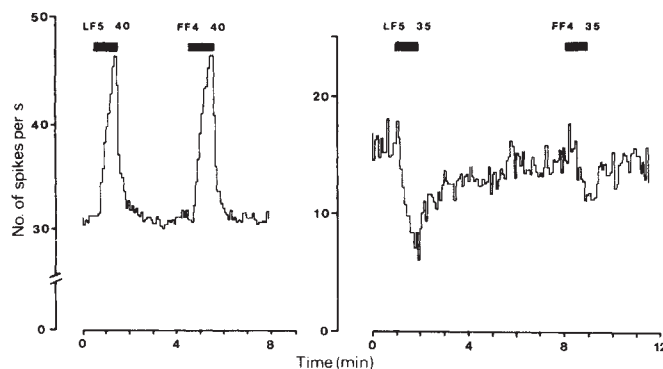
A sample of purified IIc material, estimated by radioimmunoassay to contain 22 pmol when read from the FMRFamide



**Fig. 1** Separation on CM Sephadex (lower panel) and Sephadex G-25 (upper panel) of chicken brain FMRFamide-like immunoreactivity. Chicken heads were obtained from a local abattoir and transported to the laboratory packed in ice. Brains were quickly dissected and stored at -20 °C. Tissue was extracted by adding small pieces to boiling water; extracts (100 g batches, 0.1 g ml<sup>-1</sup>) were boiled for 5 min, cooled and acidified by addition of acetic acid to 0.5 M. In extracts that were not acidified the recovery of immunoreactivity was only 60%. Extracts were centrifuged (2,000g, 20 min, 4 °C) and the supernatant was passed through Sep-pak cartridges (Waters Associates) preequilibrated with 50% acetonitrile in 0.1% trifluoroacetic acid (TFA), and washed with 0.1% TFA. On a single pass, 35-80% of the immunoreactivity in 100 ml of extract was adsorbed; routinely extracts were passed twice, eluted with 2.0 ml 50% acetonitrile and the procedure was repeated. Eluates were lyophilized, redissolved in 0.05 M ammonium acetate and applied to the CM Sephadex column (1×10 cm) which was eluted with a gradient (starting at arrow) from 0.05 M ammonium acetate pH 4.5 to 0.5 M ammonium acetate pH 7.0 (recovery of immunoreactivity was 99.5±2.6%, mean ±s.e. in eight typical runs). Peak II material was concentrated on Sep-pak cartridges using the method described above and then product fractionated by gel filtration on Sephadex G-25 (1×100 cm, in 0.5 M acetic acid, 4 °C). The recovery in four typical runs was 91.5±13.0% and the peak IIc material was taken for HPLC studies. FMRFamide-like immunoreactivity (●—●) was detected by radioimmunoassay as previously described<sup>11,15</sup>; A<sub>280</sub> is indicated by (○—○).



**Fig. 2** HPLC separation on a C18 Vydac column (5×250 mm) of peak IIc material (upper panel) and synthetic LPLRFamide prepared by CRB Ltd (lower panel). An Altex HPLC system was used and the column eluted with a gradient of acetonitrile in 0.1% TFA (as indicated by the broken line). Immunoreactivity in the IIc fraction is indicated by the hatched columns. The peak of material with A<sub>214</sub> and a retention time of 15 min is the only immunoreactive component in the synthetic preparation.



**Fig. 3** Records of single neurone activity in rat brain stem. Extracellular action potentials from spontaneously active cells were recorded through the central barrel of a multi-barrel micropipette, amplified and counted over successive 5-s periods. Peptides were administered by iontophoresis from the outer barrel of the pipette using a Neurophore BH-2 iontophoresis system with automatic current balancing. Horizontal bars indicate applications; the numbers indicate the ejecting current in nA. Retaining currents of 5–10 nA were passed between applications. The peptides were contained in the following solutions: LPLRFamide (15 mM, pH 5.0), FMRFamide (2 mM, pH 5.5), NaCl (4 M) for the recording barrel and Potamine Sky-Blue (2.5% in sodium acetate pH 5.6) for current-balancing and marking of recording sites. In the left panel a medullary reticular neurone is shown to be excited by LPLRFamide (LF5) and FMRFamide (FF4), and in the right panel a neurone in the nucleus of the solitary tract is shown to be depressed by LPLRFamide and FMRFamide.

standard curve, subsequently yielded 175 pmol Phe-NH<sub>2</sub> after digestion by trypsin, indicating that the immunochemical potency of IIC relative to the standard FMRFamide was 0.12. Microsequence analysis of ~1.0 nmol of the peptide using the method of Shively and co-workers<sup>18,19</sup> gave the sequence of Leu-Pro-Leu-Arg-?, with an N-terminal yield of 330 pmol and no positive identification of the C-terminal residue. Taken together, the data indicate the structure Leu-Pro-Leu-Arg-Phe-NH<sub>2</sub> (LPLRFamide in the single-letter notation). Synthetic LPLRFamide was subsequently found to have an immunochemical potency of 0.09 relative to FMRFamide, which is in good agreement with the findings already mentioned. The same preparation of synthetic LPLRFamide eluted in an identical position to the natural material on HPLC (Fig. 2). Approximately 0.3 nmol of immunoreactive material (FMRFamide standard) were obtained from 1.5 kg of brain. Allowing for the immunochemical potency of LPLRFamide in the present radio-immunoassay, the true concentration would be 2–3 nmol kg<sup>-1</sup>. Overall recovery was estimated to be ~40%, which indicates the presence of 5–8 nmol kg<sup>-1</sup> in whole chicken brain, although it is probable that in particular regions the concentration is much higher.

FMRFamide has been reported to increase arterial blood pressure in the urethane-anaesthetized rat<sup>7</sup> and to excite rat brain stem neurones<sup>6</sup>. The effects of LPLRFamide were therefore compared with those of FMRFamide in these systems. Intravenous injection of LPLRFamide (50–200 nmol kg<sup>-1</sup>) in urethane-anaesthetized rats produced a rapid, short-lived (<2 min) increase in arterial blood pressure with a time course similar to that of FMRFamide. Following 100 nmol kg<sup>-1</sup> LPLRFamide, the peak increase in arterial blood pressure was 16.5 ± 1.4 mm Hg (mean ± s.e., *n* = 24), compared with 15.0 ± 4.7 (*n* = 4) mm Hg following the same dose of FMRFamide. The same dose of LPLRFamide produced a longer-lasting (up to 10 min) increase in arterial blood pressure when given intracisternally (peak increase 21.8 ± 1.5 mm Hg, *n* = 12). Intracisternal injections of LPLRFamide at intervals greater than 1 h gave dose-related

effects. However, when the interval between doses was less than 1 h there was tachyphylaxis following intracisternal, although not intravenous, injection. Both intracisternal and intravenous effects were blocked by phentolamine (200 nmol kg<sup>-1</sup> h<sup>-1</sup>), suggesting that LPLRFamide acts by releasing noradrenaline from sympathetic nerve endings.

When LPLRFamide was applied by microiontophoresis to spontaneously active brain stem neurones in the rat, using similar experimental procedures to those previously reported<sup>6</sup>, its actions closely resembled those of FMRFamide (Fig. 3). On 14 of 20 medullary reticular neurones in 5 rats the effects of LPLRFamide and of FMRFamide were predominantly excitatory. However, in an adjacent area of the brain stem, the nucleus of the solitary tract, both LPLRFamide and FMRFamide had inhibitory actions (Fig. 3) on all four cells tested. No cells were found which responded to one but not the other peptide; neither were examples found of both types of response by the same cell.

Aside from the C-terminal dipeptide amide, -Arg-Phe-NH<sub>2</sub>, which is identical to that in FMRFamide and bovine  $\gamma_1$ -MSH (melanocyte stimulating hormone) (ref. 20), the pentapeptide isolated from chicken brain does not resemble any known neuropeptide. The pancreatic polypeptide group of molecules, which includes the neuropeptide NPY, terminate in -Arg-Tyr-NH<sub>2</sub> (refs 21, 22). One of these, chicken pancreatic polypeptide (APP), reacts weakly with some FMRFamide antisera<sup>15</sup>. However, there are no other similarities between LPLRFamide and known members of the PP group, for example, PYY and NPY. We therefore suggest that LPLRFamide is a representative of a new group of vertebrate neuropeptides with putative neurotransmitter or modulatory functions. It may well be that some or all of the other FMRFamide-immunoreactive species found in chicken brain represent N-terminally extended forms of LPLRFamide, and these could conceivably be the physiologically important forms of this group of molecules; in preliminary studies one peptide in the IIB fraction has been found to terminate in -Arg-Phe-NH<sub>2</sub>. The relationships between LPLRFamide and the molluscan cardio-excitatory peptides remain to be established, but the presently available sequence information cannot be regarded as compelling evidence for a common evolutionary history of this group of molecules. These results therefore emphasize the importance of full chemical characterization of molecules previously identified only by their immunochemical properties.

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## Heat shock proteins, first major products of zygotic gene activity in mouse embryo

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In many species, the early post-fertilization development of the egg appears to occur mainly under maternal control and does not require transcription of the embryonic genome<sup>1</sup>. In the mouse this situation is restricted to the one-cell stage; activation of the embryonic genome occurs at the late two-cell stage<sup>2-4</sup> and results in a drastic change in the spectrum of proteins synthesized. This activation is preceded by a decrease in the overall synthesis of proteins at the end of the one-cell stage<sup>5-7</sup> and the appearance, at the early two-cell stage, of a set of new polypeptides of molecular weight ~70,000 (70K) (refs 2, 8, 9). This can be compared with the series of events that occur after hyperthermia in differentiated cells<sup>10-13</sup>. Heat shock results in an arrest of most transcription and translation; subsequently, expression of a limited set of genes, the heat shock genes, precedes the overall reactivation of cellular genome. Here we show that the 70K early two-cell-specific proteins are identical to two of the mouse heat shock proteins, HSP 68 and HSP 70.

Two-dimensional gel electrophoretic analysis of <sup>35</sup>S-methionine-labelled proteins shows that the patterns of proteins synthesized in one-cell and early two-cell mouse embryos are similar (Fig. 1). Among the differences, however, is the appearance of the 70K proteins at the two-cell stage. The two predominant spots in this group migrate at the same point in two-dimensional gels as the HSP 68 and HSP 70

proteins synthesized in F9 cells<sup>14</sup> after exposure to sodium arsenite (Fig. 2). Like hyperthermia, sodium arsenite induces HSP synthesis in mammalian cells. Radioactive spots corresponding to HSP 68, HSP 70 and their counterparts from early two-cell embryos were cut out of the gels and subjected to limited proteolysis according to Cleveland *et al.*<sup>15</sup>. Comparison of the peptide patterns indicates that the major spots which appear at the early two-cell stage correspond to HSP 68 and HSP 70 (Fig. 2).

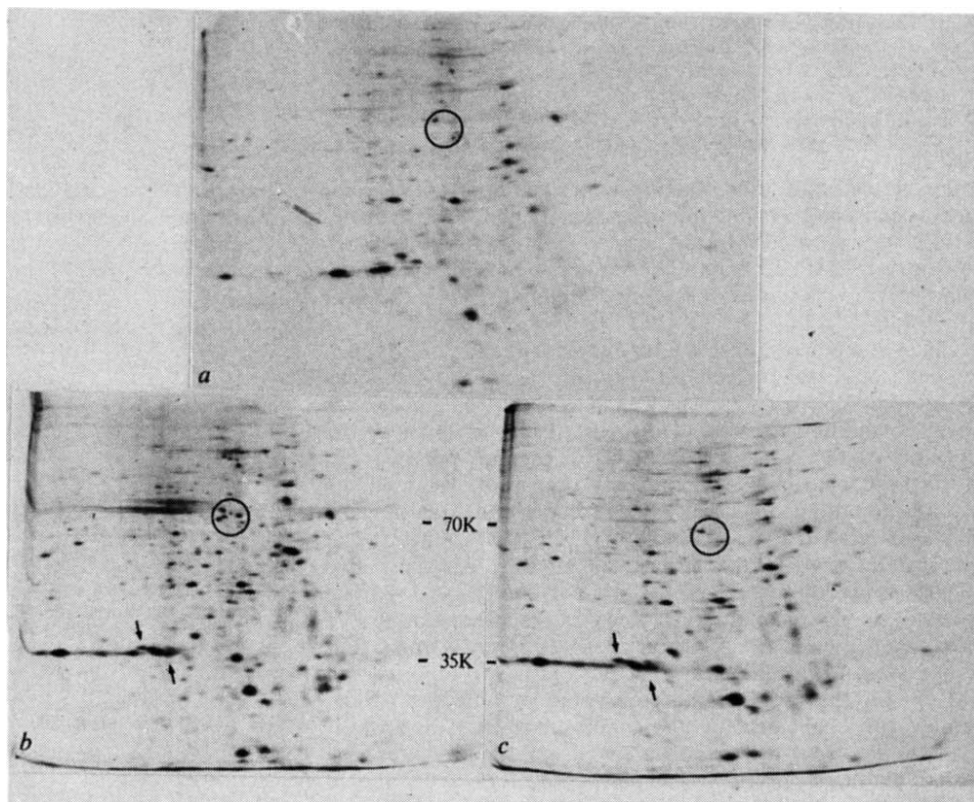
Fertilized oocytes incubated with  $\alpha$ -amanitin divide normally into two-cell embryos<sup>3,4,16</sup>. The two-dimensional pattern of proteins synthesized by such embryos is almost the same as that of untreated early two-cell embryos (Fig. 1). However, the main difference is the absence of HSP 68 and HSP 70 synthesis. A similar observation has been reported by Flach *et al.*<sup>2</sup> for the two-cell-specific 67K protein cluster. This group of proteins probably corresponds to the heat shock proteins we have identified. As  $\alpha$ -amanitin is known to inhibit mRNA synthesis in the mouse embryo<sup>17</sup>, it is likely that heat shock genes are the first genes to be efficiently transcribed after fertilization.

In many other organisms, proteins which might correspond to heat shock proteins appear during early embryogenesis concomitant with the onset of transcription of the embryonic genome. For example, in *Drosophila* this has been described at the blastoderm stage<sup>18,19</sup>. Re-examination of previously published two-dimensional gels shows that 70K proteins having isoelectric points near 5.5 appear in the rabbit 16-cell<sup>20</sup> embryo and in *Xenopus laevis* pre-blastula between the first and the sixth cleavage<sup>21</sup>. In *Xenopus* oocytes, however, regulation of heat shock protein synthesis seems to differ from that in mouse eggs. These oocytes contain appreciable levels of 'masked' HSP 70 mRNAs which become translated after hyperthermia even in the presence of  $\alpha$ -amanitin<sup>22</sup>.

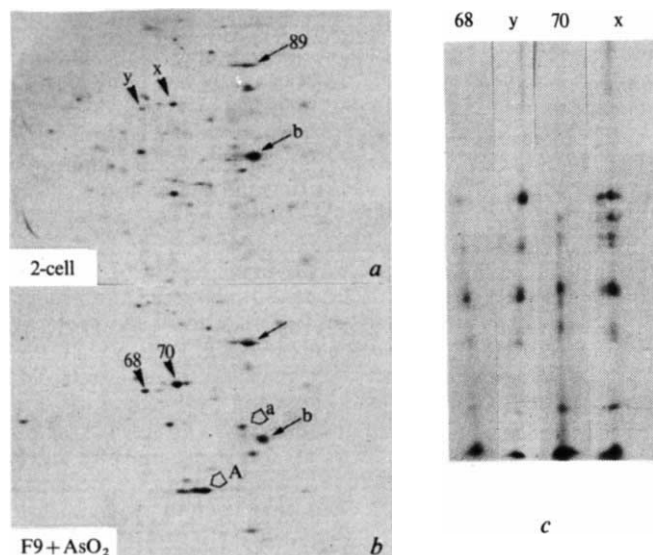
Although we found that HSP 70 was not synthesized at the time of fertilization, it might have been present after accumulation during oogenesis. To check this, the two-dimensional gels were silver-stained, and Fig. 3 shows that the silver-staining pattern is very different from the corresponding autoradiograph

**Fig. 1** Two-dimensional gel analysis of the incorporation of <sup>35</sup>S-methionine into one-cell embryos (a), two-cell embryos (b) and two-cell embryos after  $\alpha$ -amanitin treatment (c).  $\alpha$ -Amanitin inhibits the synthesis of the 70K protein cluster (in the circles) but does not prevent the appearance of the newly synthesized 35K proteins (arrows).

**Methods:** Embryos were recovered from superovulated (C57BL  $\times$  CBA) F<sub>1</sub> females mated with (C57BL  $\times$  CBA) F<sub>1</sub> males. Fertilization was assumed to have occurred 12 h post-human chorionic gonadotropin (HCG) injection and eggs were collected at 16 h. Cumulus cells were removed by hyaluronidase treatment. These operations were performed in PB1 medium in the presence of 0.3% w/v bovine serum albumin<sup>28</sup>. Eggs were then washed and cultured in Whitten's medium at 37 °C under 5% CO<sub>2</sub> in air<sup>29</sup>. a, 15 one-cell eggs were placed in label at 18 h post-HCG; b, 14 early two-cell eggs were placed in label at 37 h post-HCG; c, 29 one-cell eggs were cultured in the presence of  $\alpha$ -amanitin (12  $\mu$ g ml<sup>-1</sup>). From these, 19 which had cleaved were placed in label at 37 h post-HCG. After 3 h of labelling in <sup>35</sup>S-methionine (1 mCi ml<sup>-1</sup>)-containing medium, embryos were washed in phosphate-buffered saline, lyophilized, dissolved in O'Farrell lysis buffer and submitted to two-dimensional gel electrophoresis<sup>30</sup> (ampholines LKB: 0.4%, pH 4-6; 1.6%, pH 3.5-10). The gels were dried and autoradiographed.



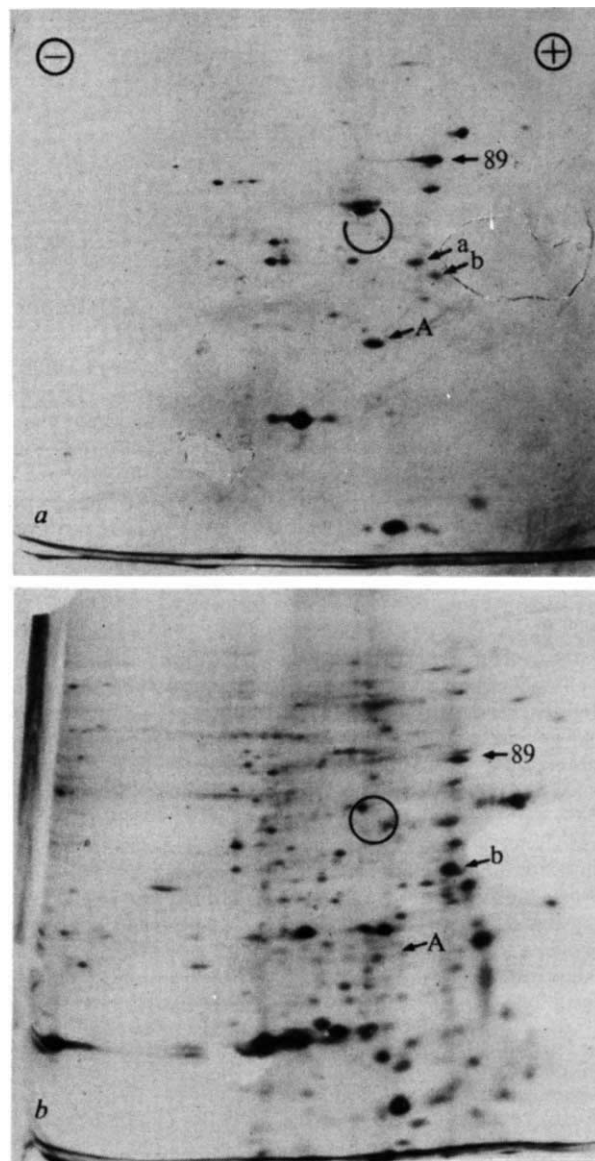




**Fig. 2 (Above)** The 70K two-cell specific proteins, x and y, have the same electrophoretic mobilities and the same pattern of peptides after limited proteolysis as HSP 68 and HSP 70 (arrows). An autoradiogram of a two-dimensional gel of early two-cell embryo proteins (a) is compared with that of sodium arsenite-treated F9 mouse cells (b). c, Peptide patterns of HSP 68 and HSP 70 from arsenite-treated F9 cells are compared with those of the corresponding two-cell specific proteins, x and y.

**Methods:** Embryos were processed as described in Fig. 1 legend, F9 cells were exposed to 150  $\mu$ M arsenite for 45 min. After 2 h of recovery in standard Eagle's medium supplemented with 15% fetal calf serum, cells were labelled during 3 h in methionine-free medium, dissolved in O'Farrell lysis buffer and submitted to two-dimensional gel electrophoresis (ampholines LKB: 1.6%, pH 4–6; 0.4%, pH 3.5–10). Radioactive spots were cut out of the gels, partially digested with *Staphylococcus aureus* V8 protease and analysed electrophoretically according to Cleveland *et al.*<sup>15</sup>.

**Fig. 3 (Right)** The presence of abundant proteins like HSP 89 (89), Actin (A),  $\alpha$ -tubulin (a) or  $\beta$ -tubulin (b) in one-cell embryos as demonstrated by silver staining of the two-dimensional gel (a), but incorporation of <sup>35</sup>S-methionine into actin and  $\alpha$ -tubulin is hardly detectable on the autoradiograph (b). In contrast, HSP 70 cannot be revealed by either method. For this experiment, 75 one-cell embryos were processed as in Fig. 1. The gel was silver-stained<sup>31</sup> before drying and autoradiography.



at the one-cell stage. For example, although important proteins such as actin and  $\alpha$ -tubulin are heavily stained, little radioactivity is incorporated into the corresponding spots (Figs 2, 3). In contrast, HSP 70 cannot be detected by either method, demonstrating that one-cell embryos contain none (or very little) of it.

The heat shock response is a very general phenomenon which has been observed for cells of various origins<sup>10,11,13</sup>, and can be induced by a wide range of aggressions or chemicals (for example, sodium arsenite). However, it always involves the synthesis of the same set of highly conserved polypeptides whose function remains unknown. These proteins might be required for the recovery of gene expression in heat-shocked cells<sup>12</sup>. In most cell types studied, HSP 70 is synthesized at appreciable levels in the absence of stress<sup>23</sup>. However, two exceptions are known, the mouse one-cell embryo as described here and the mammalian erythrocyte<sup>24</sup>. It is interesting that in these two cell types transcription does not occur. It might thus be necessary for the embryo to acquire rapidly a high level of HSP 70 for the onset of the embryonic genome expression. Perhaps this could explain why conditions such as hyperthermia<sup>25</sup>, or exposure to ethanol<sup>26,27</sup>, which stimulate heat shock protein synthesis, promote the parthenogenetic activation of the mouse oocytes.

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## Regulated expression of the human $\beta$ -globin gene family in murine erythroleukaemia cells

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**Chemically induced differentiation of cultured murine erythroleukaemia (MEL) cells results in a several hundred-fold increase in transcription of the adult mouse globin genes<sup>1–3</sup> and thus serves as a model for gene activation during erythropoiesis. One approach to study gene regulation in this system has been to analyse the expression of foreign globin genes introduced into MEL cells<sup>4–8</sup>. By introducing cosmid DNA containing the human adult( $\beta$ ), fetal( $\gamma$ ) and embryonic( $\epsilon$ )-globin genes, we have shown here that expression of the  $\beta$ , but not the  $\gamma$  or  $\epsilon$  genes, is regulated during MEL differentiation. Regulated expression of the human  $\beta$ -globin gene was observed when it was introduced either as part of the intact globin gene cluster or as an individual gene with 1.5 kilobases (kb) of 5' flanking DNA. Transcription from a herpes simplex virus (HSV) promoter adjacent to the thymidine kinase (*tk*) gene is also inducible in MEL cells.**

Cosmids containing regions of the human  $\beta$ -globin gene cluster (see Fig. 1) were introduced into thymidine kinase-negative (*tk*<sup>-</sup>) MEL cells<sup>6</sup> by calcium phosphate transformation<sup>9</sup>. Before transformation, DNA was linearized within the vector sequences by cleavage with *PvuI*. Stable *tk*<sup>+</sup> transformants were obtained at a frequency of 1–10 clones per 10<sup>6</sup> cells per  $\mu$ g DNA. Transformants were shown by Southern blot analysis<sup>10</sup> to contain 1–15 copies of non-rearranged introduced DNA per cell (data not shown); 10–15 transformants containing each cosmid were induced into erythroid differentiation by cul-

turing for 3 days in the presence of 3 mM hexamethylene bisacetamide (HMBA). Levels of human and mouse globin transcripts were quantitated by S<sub>1</sub> nuclease analysis<sup>11,12</sup> using probes which mapped the 5' and 3' ends of the mRNAs.

Endogenous mouse  $\beta$ -major globin mRNA levels in transformants as measured by S<sub>1</sub> nuclease analysis of the 3' end of the mRNA are shown in Table 1 and Fig. 2a; 52 of 54 clones tested showed a >100-fold increase in mouse  $\beta$ -globin mRNA levels on differentiation, corresponding to >10,000 copies of mRNA per induced cell. Six of the transformants which showed induction had a high level of mouse  $\beta$ -major globin mRNA before HMBA treatment (>1,000 copies per cell).

Of 40 MEL transformants that contained a foreign human  $\beta$ -globin gene, 30 showed a 4–100-fold increase in the level of human  $\beta$ -globin mRNA on differentiation as measured by S<sub>1</sub> nuclease analysis of the 3' end of the mRNA (Fig. 2b, c; Table 1). Of the 10 transformants that showed no induction, two were non-inducible for endogenous mouse  $\beta$ -globin mRNA (one CosHG6N and one  $\beta$ -pRT) and four showed a high level of mouse globin mRNA before addition of the inducing agent (two CosHG6N and two CosHG28TK). Correct initiation of the human globin transcripts was shown by S<sub>1</sub> nuclease mapping of the 5' end (Fig. 2d). Induced human  $\beta$ -globin mRNA levels corresponded to 120–2,500 copies per cell, and induction levels did not correlate with the copy number of human  $\beta$ -globin genes present in the transformant.

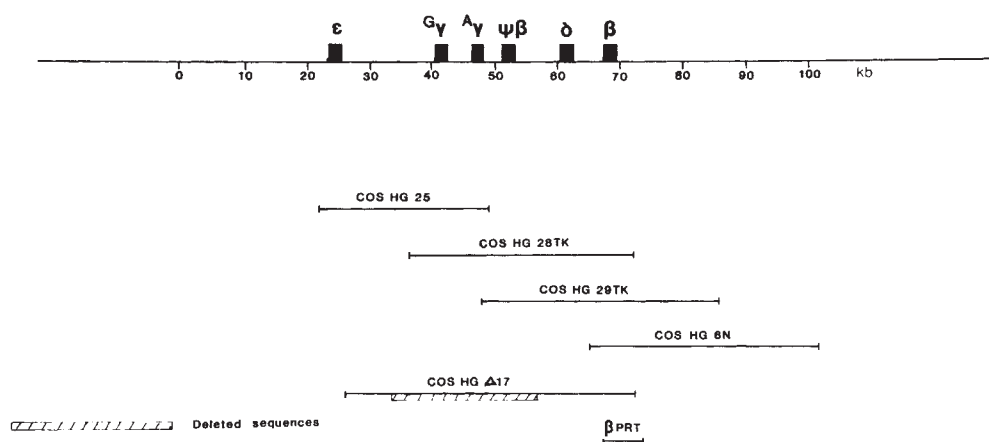
Of 19 transformants containing the complete human  $\gamma$ -globin genes (CosHG25 and CosHG28TK), 17 showed the same or a smaller amount of  $\gamma$ -globin mRNA after differentiation (~50 copies per cell; Fig. 2e, Table 1). Two transformants showed a less than sixfold inducibility of  $\gamma$ -globin mRNA. Similarly, transformants which contained the complete  $\epsilon$ -globin gene (CosHG25) showed the same or reduced levels of  $\epsilon$ -globin mRNA after differentiation (data not shown).

CosHG29TK and CosHG $\Delta$ 17 contain the 3' ends of the A $\gamma$ - and  $\epsilon$ -globin genes adjoined to vector sequences as shown in Figs 1 and 3. Surprisingly, transformants containing these cosmids showed a 10–30-fold inducibility of RNAs which were detected by S<sub>1</sub> nuclease analysis using probes which hybridized to the 3' ends of A $\gamma$ - and  $\epsilon$ -globin mRNAs. Northern blot analysis<sup>13</sup> showed that the inducible RNA was 700 base pairs (bp) long and that it hybridized with both vector and globin DNA probes (not shown). If we assume the only RNA splicing in this vector-globin hybrid mRNA to be removal of the globin large intervening sequence, the observed RNA length places the transcription initiation site close to that of the HSV *tk* gene<sup>14</sup>, but on the opposite DNA strand. This was confirmed

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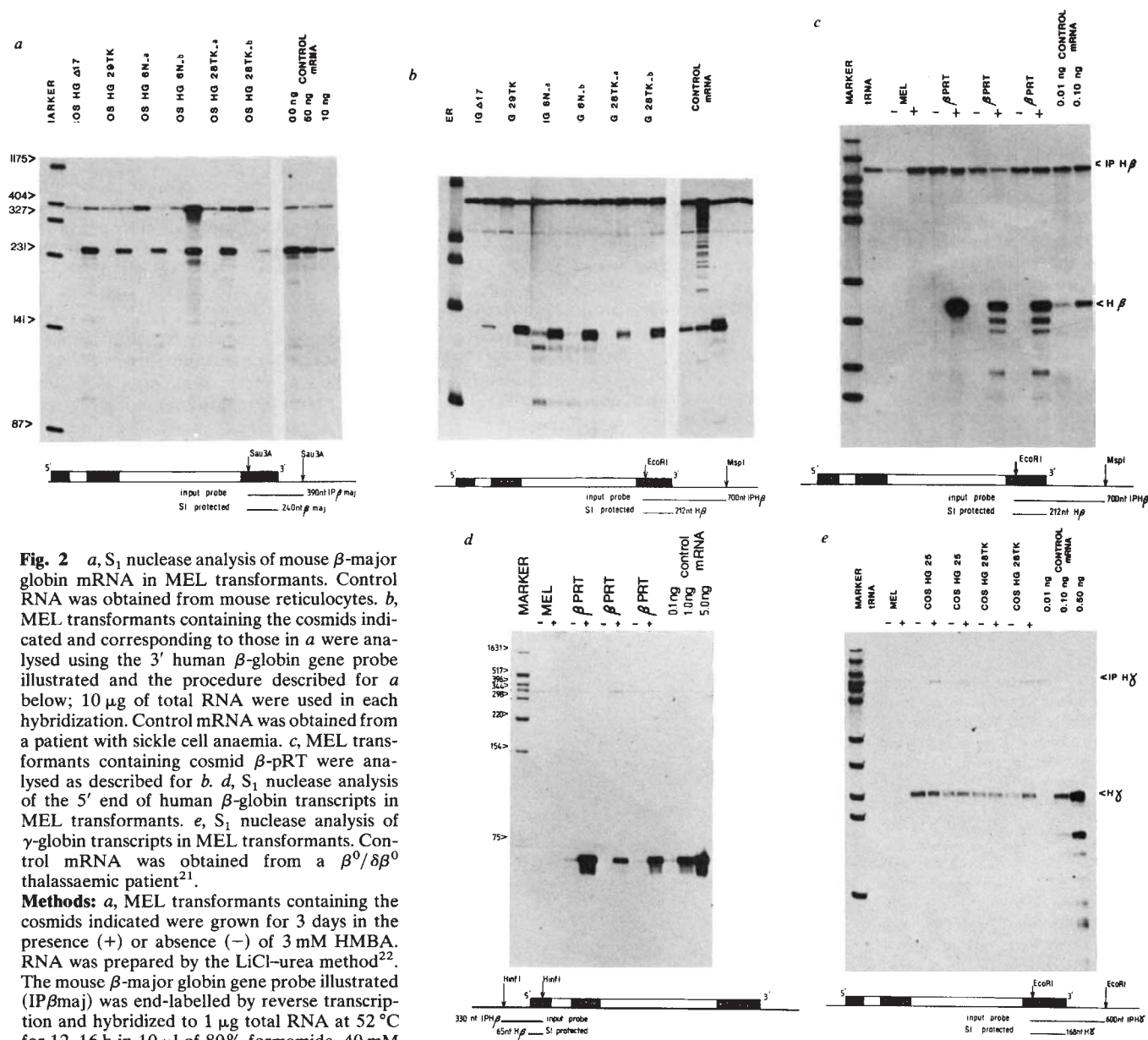
**Fig. 1** Structure of human globin gene cosmids introduced into MEL cells. Regions of the human globin cluster as indicated were cloned in cosmid vectors containing the HSV *tk* gene<sup>17,18</sup> (vector sequences not shown). CosHG25 and CosHG6N were isolated directly from a *tk* vector library. CosHG28TK and CosHG29TK were constructed from previously isolated cosmids, CosHG28 and CosHG29 (ref. 18) by the exchange of vector sequences containing the *tk* gene. CosHG $\Delta$ 17 was constructed by packaging and transduction of a ligation mixture containing a *ClaI*-*Bam*HI fragment from cosmid pRT, a *Bam*HI-*Xma*I fragment containing the 3' side of the  $\epsilon$ -gene, and *Xma*I-*Pvu*I fragment containing the G $\gamma$ -, A $\gamma$ -,  $\delta$ - and  $\beta$ -globin genes and part of the cosmid vector pJB8 (ref. 19). Ligation yielded a DNA concatemer which deleted during the *in vitro* packaging to give deletion cosmids including CosHG $\Delta$ 17.  $\beta$ -pRT is a subclone of the 4.7-kb  $\beta$ -globin *Bga*I fragment inserted in the *Bam*HI site of vector pRT; the *tk* and  $\beta$ -globin genes in this construction are transcribed in the same direction. Cosmid DNA was prepared by the method of Birnboim and Doly<sup>20</sup> followed by purification on CsCl gradients. *Pvu*I-linearized cosmid DNA was introduced into *tk*<sup>-</sup> MEL cells<sup>6</sup> by calcium phosphate transformation<sup>8</sup> and selection in hypoxanthine-aminopterin-thymidine medium.



**Table 1** Induction of human and mouse globin mRNAs in cosmid-containing MEL transformants

| Cosmid            | Mouse $\beta$ -major globin mRNA |                       |                                  | Human $\gamma$ -globin mRNA |                       |                          | Human $\beta$ -globin mRNA |                       |                          |
|-------------------|----------------------------------|-----------------------|----------------------------------|-----------------------------|-----------------------|--------------------------|----------------------------|-----------------------|--------------------------|
|                   | No. of clones tested             | No. showing induction | No. with high non-induced levels | No. of clones tested        | No. showing induction | No. showing no induction | No. of clones tested       | No. showing induction | No. showing no induction |
| CosHG25           | 9                                | 9                     | 2                                | 9                           | 0                     | 9                        | —                          | —                     | —                        |
| CosHG $\Delta$ 17 | 8                                | 8                     | 0                                | —                           | —                     | —                        | 8                          | 6                     | 2                        |
| CosHG28TK         | 10                               | 10                    | 2                                | 10                          | 2                     | 8                        | 10                         | 7                     | 3                        |
| CosHG29TK         | 9                                | 9                     | 0                                | 9*                          | 6*                    | 3*                       | 4                          | 4                     | 0                        |
| CosHG6N           | 8                                | 7                     | 2                                | —                           | —                     | —                        | 8                          | 5                     | 3                        |
| $\beta$ -pRT      | 10                               | 9                     | 0                                | —                           | —                     | —                        | 10                         | 8                     | 2                        |
| Total             | 54                               | 52                    | 6                                | 19                          | 2                     | 17                       | 40                         | 30                    | 10                       |

\* Transcription of the  $\gamma$ -globin gene in MEL clones containing CosHG29TK initiates at a vector promoter (see text) and is not included in the totals column.

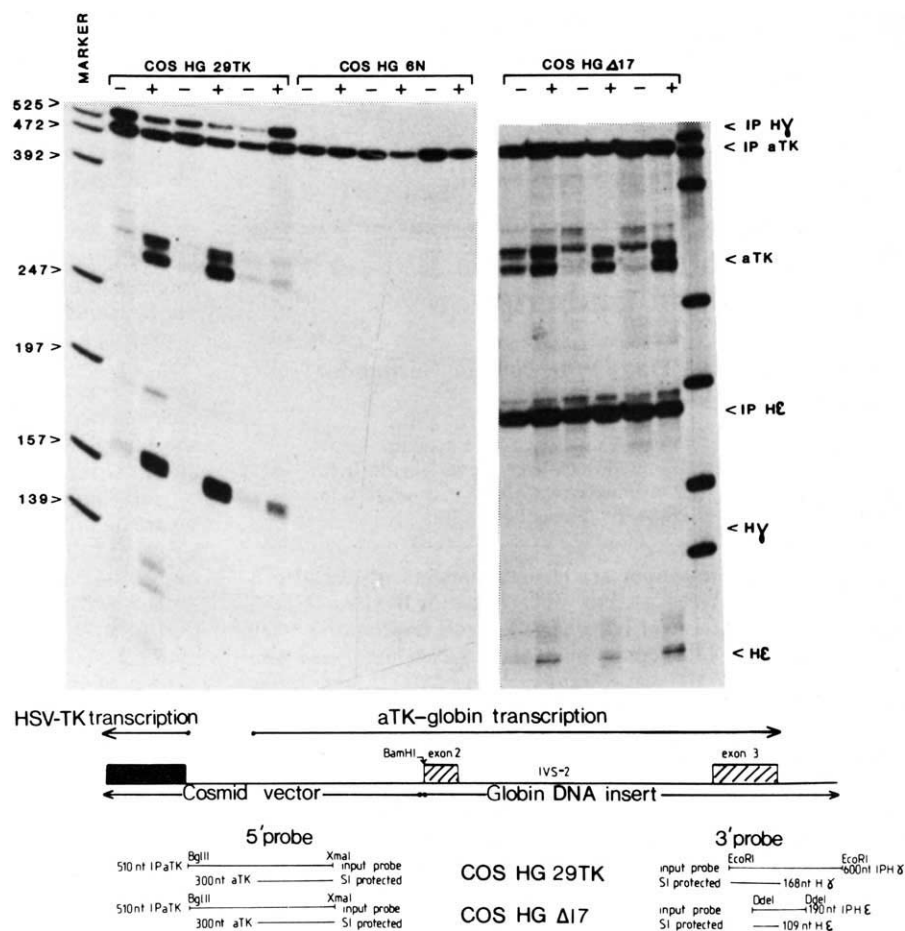


**Fig. 2** *a*, S<sub>1</sub> nuclease analysis of mouse  $\beta$ -major globin mRNA in MEL transformants. Control RNA was obtained from mouse reticulocytes. *b*, MEL transformants containing the cosmids indicated and corresponding to those in *a* were analysed using the 3' human  $\beta$ -globin gene probe illustrated and the procedure described for *a* below; 10  $\mu$ g of total RNA were used in each hybridization. Control mRNA was obtained from a patient with sickle cell anaemia. *c*, MEL transformants containing cosmid  $\beta$ -pRT were analysed as described for *b*. *d*, S<sub>1</sub> nuclease analysis of the 5' end of human  $\beta$ -globin transcripts in MEL transformants. *e*, S<sub>1</sub> nuclease analysis of  $\gamma$ -globin transcripts in MEL transformants. Control mRNA was obtained from a  $\beta^0/\delta\beta^0$  thalassaemic patient<sup>21</sup>.

**Methods:** *a*, MEL transformants containing the cosmids indicated were grown for 3 days in the presence (+) or absence (−) of 3 mM HMBA. RNA was prepared by the LiCl-urea method<sup>22</sup>. The mouse  $\beta$ -major globin gene probe illustrated (IP $\beta$  maj) was end-labelled by reverse transcription and hybridized to 1  $\mu$ g total RNA at 52 °C for 12–16 h in 10  $\mu$ l of 80% formamide, 40 mM PIPES pH 6.4, 1 mM EDTA, 400 mM NaCl. The mixture was digested with 3,000 units of S<sub>1</sub> nuclease (Boehringer) in 300  $\mu$ l of 300 mM NaAc pH 4.8, 200 mM NaCl, 2 mM ZnSO<sub>4</sub> for 2 h at 20 °C. S<sub>1</sub>-protected DNA was ethanol-precipitated and electrophoresed on a 7 M urea, 7% acrylamide gel. *d*, MEL transformants containing cosmid  $\beta$ -pRT and corresponding to those used in *c* were analysed. The procedure was as for *a* except that the 5' human  $\beta$ -globin probe was end-labelled by kinase, strand-separated, and hybridized to 120  $\mu$ g total RNA in a 50  $\mu$ l hybridization volume. *e*, RNA from MEL transformants containing the cosmids indicated was analysed using the  $\gamma$ -globin gene probe illustrated and the procedure described for *a*. 10  $\mu$ g of total RNA were used in each hybridization.



**Fig. 3**  $S_1$  nuclease analysis of anti-*tk* transcripts in MEL transformants. MEL transformants containing cosHG29TK, cosHG6N and cosHG $\Delta$ 17 were grown for 3 days in the presence (+) or absence (-) of 3 mM HMBA. CosHG29TK and cosHG $\Delta$ 17 contain the 3' ends of the  $\gamma$ - and  $\epsilon$ -globin genes adjoined to vector sequences near the HSV *tk* gene as shown. The 5' ends of *a-tk* transcripts initiating near the *tk* gene were mapped with a 510 bp *Xma*I-*Bgl*II probe (IP aTK) using the procedure described for Fig. 2a. This probe covers the expected 5' end of the *a-tk* transcript and gives an  $S_1$  nuclease-protected fragment of 280–300 nucleotides (aTK). RNA from cosHG29TK and cosHG $\Delta$ 17 transformants was simultaneously hybridized to 3'  $\gamma$ -globin and 3'  $\epsilon$ -globin gene probes, respectively.



by  $S_1$  nuclease mapping using a 510-bp *Xma*I-*Bgl*II probe which covers the predicted 5' end and gives a 280–300-bp protected band. The 5' end of this 'anti-*tk*' (*a-tk*) transcript thus maps ~160 bp away from the *tk* gene cap site (see Fig. 3). A transcript initiating at this site has been previously reported from *in vitro* transcription of HSV DNA fragments<sup>15</sup>. ATA and CAAT box sequences required for transcription initiation are located at the appropriate distances 5' to the *a-tk* transcript. When this promoter is not linked to known coding sequences (in CosHG25, CosHG28TK and CosHG6N), no transcripts originate from it (Fig. 3, centre panel), suggesting that either an RNA splice or transcription terminator are required to yield a stable transcript.

We have shown independent regulation in the expression of individual human  $\epsilon$ -,  $\gamma$ - and  $\beta$ -globin genes when introduced into MEL cells as a gene cluster, in accordance with previous work by Willing *et al.*<sup>4</sup> and Pyati *et al.*<sup>5</sup> who used human chromosome 11-MEL cell hybrids. Expression of human adult, but not fetal or embryonic globin genes, is regulated during MEL cell differentiation. This, therefore, mimics the specific activation of adult-type globin genes during erythropoiesis in man. Regulated expression of the human  $\beta$ -globin gene is independent of the presence of the rest of the globin gene cluster and is observed when it is introduced with only 1.5 kb of 5' flanking DNA ( $\beta$ PR; Fig. 2c). We have not directly excluded the possibility that the increased globin mRNA levels found after MEL cell differentiation are due to changes in mRNA stability as opposed to changes in transcription rate, although strong arguments suggest that this is not the case. First, the human  $\epsilon$ - and  $\gamma$ -globin mRNAs are constitutively produced in MEL cells and in some exceptional clones even  $\beta$ -globin mRNA is constitutive. This suggests that  $\epsilon$ - and  $\gamma$ -globin mRNAs are stable in uninduced MEL cells and therefore makes it unlikely that  $\beta$ -globin mRNA is unstable. Note that  $\gamma$ - and  $\beta$ -globin mRNAs stably coexist in human erythroid cells, for example, in heterozygotes for hereditary persistence of fetal haemoglobin<sup>16</sup>. Moreover, the 3' regions of the same  $\epsilon$ - and  $\gamma$ -globin

mRNAs become inducible when linked to the HSV promoter, which suggests that the induction is not related to the stability of the mRNA.

The level of induction of the introduced gene is about one order of magnitude lower than that observed for the endogenous mouse  $\beta$ -major globin gene. This lower level probably cannot be explained by the fact that the human  $\beta$ -globin gene and mouse MEL cells represent a heterologous system; Chao *et al.*<sup>8</sup> have recently shown that the transcription of a mouse-human hybrid gene is also inducible in this system. As the level of hybrid mouse-human mRNA derived from the mouse promoter is about the same as that derived from the human promoter (present results and ref. 8) a heterologous effect is unlikely. It is, therefore, probable that the lower level of induction of the foreign compared with the endogenous globin genes reflects differences in chromosomal location and chromatin structure of the introduced gene. Such differences would also explain the variable level of induction of the human  $\beta$ -globin gene between different transformants.

We were surprised to find an inducible transcript which initiated in the 5' flanking region of the HSV *tk* gene. If this viral promoter is used naturally in the herpes life cycle, it is possible that it responds to a viral or cellular *trans*-acting factor similar to that present in induced, but not in uninduced MEL cells.

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## Signals regulating hepatitis B surface antigen transcription

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About 200 million people are chronic carriers of hepatitis B surface antigen (HBsAg), but since hepatitis B virus (HBV) cannot be propagated *in vitro*, HBsAg transcription has been studied only in cell lines containing HBV DNA integrated into chromosomes, and HBsAg-related mRNAs 2.0 to 2.5 kilobases (kb) long have been described<sup>1-4</sup>. We have analysed the transcripts produced in an infected chimpanzee liver and in a rat cell line containing HBV DNA<sup>5</sup>. In contrast to previous suppositions<sup>1,2</sup> we report here that the major S gene transcript initiates close to the S gene, that is, within the 'pre-S' region<sup>6</sup> and is processed/polyadenylated at a site situated within the core gene. The efficiency of processing/polyadenylation at this site varies between the chimpanzee liver and the rat cell line studied. The S gene promoter does not contain a TATA box but instead has a sequence homologous to that which positions the 5' ends of the major simian virus 40 (SV40) late transcript<sup>7</sup>.

We extracted RNA from a liver specimen obtained from a chimpanzee infected with HBV of known sequence (refs 8, 9 and H.W. *et al.*, manuscript in preparation). However, this material was limited and contained only about 20 copies of HBV S gene transcripts per cell. Therefore we also prepared RNA from a cell line whose HBV-specific transcripts had been previously analysed by Northern hybridization<sup>2</sup> (rat 2/130.4/TK4; ref. 5), designated here as rat.HBV. As the concentration of S gene transcripts in this line is about 100 molecules per cell, this RNA was used to establish optimal conditions for the analysis of the liver RNA.

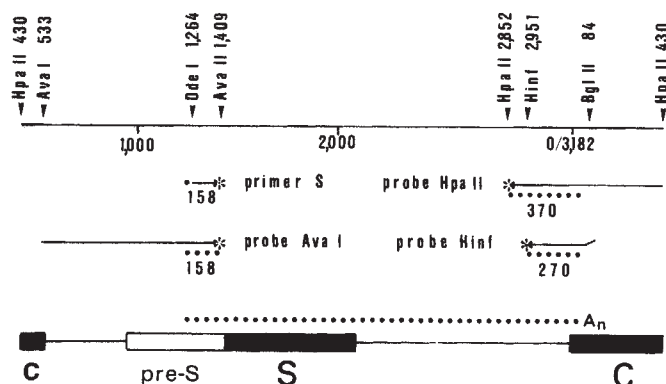
The S gene transcription unit had been defined as covering ~2.7 kb, with ~900 bp upstream of the S gene coding region<sup>1,10-12</sup>. Therefore, we used a probe covering this region (probe *AvaI*; Fig. 1, centre) in an S<sub>1</sub> protection experiment to map initiation of transcription. Figure 2A shows that only one major DNA species was protected by RNA from both sources (lanes liver×S<sub>1</sub> and rat.HBV×S<sub>1</sub>, bands marked with an asterisk). The corresponding RNA has its 5' ends at about HBV nucleotide 1,255 (Fig. 1, centre), that is, 185 nucleotides upstream of the S gene coding region. Essentially the same fragment of probe *AvaI* was protected when the RNA-DNA hybrids were digested with exonuclease VII (Fig. 2A, lanes liver×ExoVII and rat.HBV×ExoVII), which does not attack the loop formed by a genomic DNA probe when hybridizing with a spliced RNA<sup>13</sup>. These results argue against the existence of an intron in the first part of the S gene transcription unit. The 5-nucleotide difference in length between the S<sub>1</sub> and exonuclease VII digestion products is due to the fact that exonuclease VII does not digest to completion the ends of single-stranded DNA<sup>14</sup> probes.

Further evidence that the 5' end detected 185 nucleotides upstream of the S gene does correspond to a transcription initiation site comes from primer extension experiments: when primer S (Fig. 1, centre) was hybridized with RNA extracted

from the rat.HBV cell line and elongated with reverse transcriptase, only one specific band (marked with an asterisk in Fig. 2B, lane rat.HBV×primer S) was identified. As was the case for nuclease protection experiments, this band corresponds to an RNA having its 5' ends at ~185 nucleotides upstream of the S gene. Several other bands resulting from self-priming occurred also in the negative control.

As a third experimental approach to investigate the origin of the RNA species identified above, we used cloned HBV DNA<sup>15</sup> as a template in an *in vitro* transcription system in which promoter-specific initiation depends on the addition of a cytoplasmic fraction to a nuclear extract (N.H. and W. Keller, manuscript in preparation). In an S<sub>1</sub> protection experiment with probe *AvaI* (Fig. 2C), transcription initiation 185 nucleotides upstream of the S gene was observed (band marked with an asterisk in the lane labelled nuclear XT+cyt.fr.) only when the nuclear extract was complemented with the cytoplasmic fraction. Specific initiation of transcription at this site was abolished after cleavage of the putative promoter sequence about 30 nucleotides upstream of the major S start (Fig. 3, site *Fnu4H*) but not after cleavage 220 nucleotides further upstream (site *BamHI* at position 1,004; G. Christofori, N.H. and R.C., unpublished results). Thus, all sequences necessary for initiation of S gene transcription *in vitro* are encoded in the 250 nucleotides preceding the major S gene start site.

As a rule, an A+T-rich region (TATA box) responsible for correct positioning of transcription initiation<sup>16</sup> is encoded 30 nucleotides upstream of the start site of eukaryotic mRNAs. Exceptions to this rule are the SV40 and polyoma late promoters and the adenovirus 2 early region 2 promoter. Recently, Brady *et al.*<sup>7</sup> concluded that the sequence GGTACCTAACC (underlined in Fig. 3) located 30 nucleotides upstream of the SV40 major late start is important in the control of SV40 late expression. We find a very similar sequence, which is part of a region of extensive structural homology between HBV and SV40 (box in Fig. 3), in the same position relative to the major HBV S mRNA start. A second element of the SV40 late promoter was recently identified within the SV40 origin of replication<sup>14,15</sup>. Again, there is a stretch of strong sequence homology between the SV40 origin and sequences about 60 nucleotides upstream of the major S mRNA start site. These structural homologies and the fact that both SV40 late and HBV S promoters control the expression of the major viral proteins suggest that these promoters could define a novel class of regulating sequences.



**Fig. 1** Map of the HBV genome, the probes and primers used, and the transcripts detected. Top line, restriction sites used for the construction of the probes. The circular HBV genome is opened at the *HpaII* site at nucleotide 430 (convention of Pasek *et al.*<sup>24</sup>), and the position of the first nucleotide of the recognition sequence of each enzyme is indicated. Centre, probes and primers used and transcripts detected. The probes and primer S (for details see Fig. 2) are drawn as lines, the position of the label indicated with an asterisk and the RNA species protected or elongated depicted as dotted lines. Bottom, map of the HBV genome. The surface antigen (S) and the core antigen (C) genes are shown as solid boxes, the pre-S region as an open box, and the S mRNA as a dotted line.

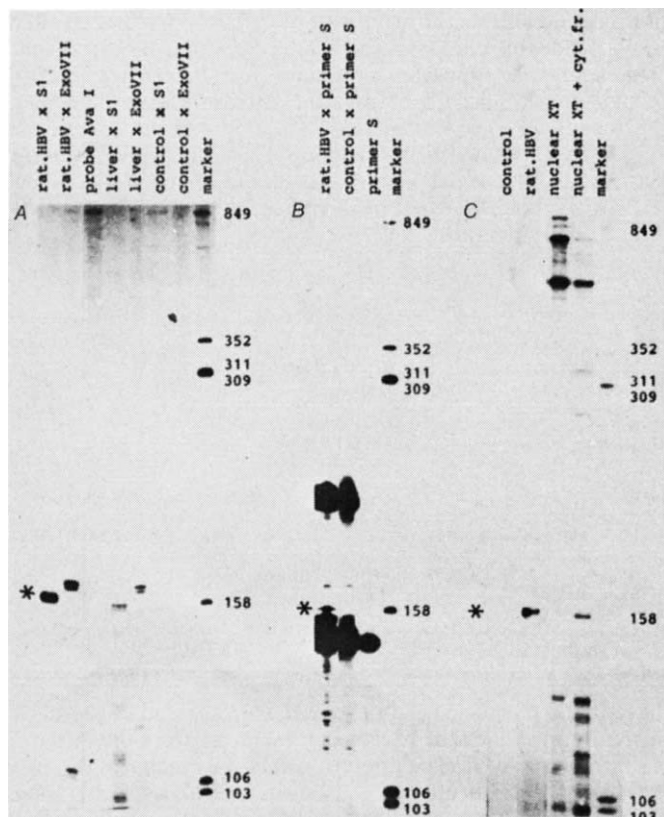


The 3' end of the *S* gene transcript was previously localized by Northern blotting about 1,000 nucleotides downstream of the *S* gene coding region<sup>1,2</sup>. To map this 3' end more precisely we used in an *S*<sub>1</sub> protection experiment a probe covering this region (probe *Hpa*II; Fig. 1, centre). Figure 4A (lanes labelled liver and rat.HBV, double band marked with 370) shows that only one DNA species was protected by the RNA from both sources. The corresponding RNA has its 3' end at HBV nucleotide 40 ± 5, that is, at the appropriate distance of 20 nucleotides

from the variant polyadenylation signal<sup>17</sup>, 16-TATAAA-21 in the HBV DNA. As this site maps within the core antigen-coding sequence, expression of functional core antigen requires transcripts which are not terminated at an AAUAAA-related sequence, as also seems to be the case for other genes<sup>18-20</sup> and for the core gene itself when expressed in SV40 vectors<sup>21</sup>.

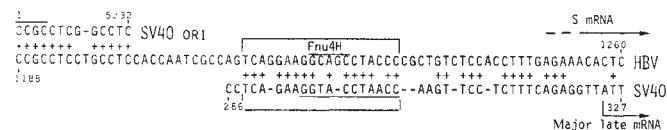
To determine the fraction of these unprocessed transcripts, we constructed a probe (probe *Hinf*; Fig. 1, centre) that was colinear with HBV DNA only for 319 of 334 nucleotides: transcripts not terminated at nucleotide 40 should protect probe *Hinf* for 319 nucleotides and processed transcripts for 270 nucleotides. Figure 4B shows that the percentage of unprocessed transcripts was low, but at least three times higher in the rat.HBV cell line than in the infected liver (compare the strength of the bands marked with an asterisk in lanes labelled liver and rat.HBV). The unprocessed transcripts represent ~10% of the terminated transcripts in the rat.HBV line and thus probably correspond to the 4.4- and 4.0-kb mRNAs detected in the same cell line and shown to hybridize with a region following the termination signal<sup>2</sup>. Processing/polyadenylation of HBV transcripts at a site situated within the core antigen gene could constitutively keep low the core/e antigen expression, and differential processing efficiency at different viral cycle stages could modulate the expression of HBsAg and 'X' proteins<sup>6</sup> relative to core/e antigens, 'polymerase' and 'pre-S'<sup>5</sup>.

The termini of the major *S* gene mRNA appear to be identical in the infected liver and in the rat.HBV cell line and their positions predict a length of 1.97 kb for an unspliced *S* gene transcript. The presence of such an unspliced mRNA has recently been demonstrated by *S*<sub>1</sub> protection experiments (R.C., unpublished results); assuming a poly(A) tail of 130 nucleotides,

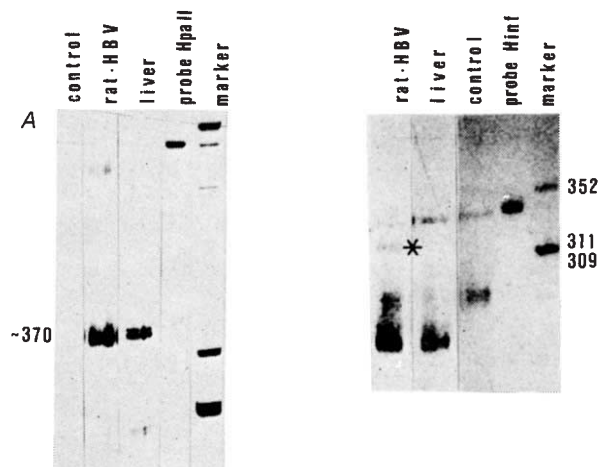


**Fig. 2** Analysis of the 5' ends of the *S* gene transcript by *S*<sub>1</sub> and exonuclease VII mapping (A), primer extension (B), and *in vitro* transcription (C). The sources of the RNAs and the nucleases used for digestion are indicated above the lanes. Negative controls (lanes marked control) were RNAs extracted from a healthy liver specimen (A), from the COS 7 cell line (B) or tRNA (C). Probe *Ava*I (A), primer S (B) and molecular weight markers were also loaded on the gels as indicated.

**Methods:** Probes *Ava*I, *Hpa*II and primer S were derived from plasmid pSHH2.1 (ref. 15). The *Ava*I probe was a 876-bp long *Ava*I fragment labelled at both 5' ends with [ $\gamma$ -<sup>32</sup>P]ATP (specific activity ~5 × 10<sup>5</sup> c.p.m. pmol<sup>-1</sup> end). Primer S was constructed by cutting probe *Ava*I with *Dde*I and isolating the 145-bp fragment. Probe *Hpa*II was a 760-bp long *Hpa*II fragment labelled by repair synthesis with [ $\alpha$ -<sup>32</sup>P]ATP at both 3' ends (specific activity ~2 × 10<sup>6</sup> c.p.m. pmol<sup>-1</sup> end). Probe *Hinf* was a 334-bp fragment from plasmid pSH2.5Δ *Bgl*II<sup>25</sup>. It is colinear with HBV DNA up to position 88, that is, for 319 nucleotides, and was labelled at both 3' ends with the same specificity as probe *Hpa*II. Nuclear and cytoplasmic extracts of HeLa cells for *in vitro* transcription were prepared by a modification of the procedure used to make whole cell extracts (ref. 26 and N.H. and W. Keller, manuscript in preparation); the template was plasmid pSH2.6<sup>15</sup>, containing a 1.12-unit length HBV genome opened at nucleotides 899 and 1,280. RNA was extracted from a liver biopsy obtained during the acute phase of hepatitis infection in a chimpanzee, from the rat.HBV cell line, and from *in vitro* reactions as described elsewhere (refs 27, 28 and 26, respectively). For each lane, RNA from 8 mg of liver tissue (~1.5 × 10<sup>6</sup> cells), from ~10<sup>6</sup> rat.HBV cells, or from 0.5 standard *in vitro* reactions<sup>26</sup> was hybridized at 51 °C with 0.02 pmol of probe *Ava*I or at 47 °C with 0.02 pmol of primer S. Hybrids were digested with *S*<sub>1</sub> nuclease or exonuclease VII or elongated with reverse transcriptase as described elsewhere<sup>13,29,30</sup>, and analysed on 6% acrylamide sequencing gels.



**Fig. 3** Alignment of nucleotides from the HBV *S* promoter (middle), the SV40 late promoter (bottom) and the SV40 origin (top). The cleavage site used to inactivate the *S* gene promoter is indicated (*Fnu*4H); the sequence important in positioning the major SV40 late transcript<sup>7</sup> is underlined; and the 4-bp deletion in the SV40 origin strongly reducing SV40 late transcription<sup>14</sup> is overlined. The SV40 major late RNA initiating at nucleotide 325 (coordinates as in ref. 31) and the *S* gene mRNA initiating at nucleotide ~1,255 (Fig. 2) are indicated. Homologous nucleotides are indicated with a + and the region of strongest homology between SV40 and HBV is boxed.



**Fig. 4** *S*<sub>1</sub> protection analysis of the 3' ends of the *S* gene transcript. RNA from infected liver or the rat.HBV cell line (as indicated in the lanes) was hybridized at 52 °C with 0.02 pmol of probe *Hpa*II (A) or 0.02 pmol of probe *Hinf* (B). Negative controls consisted of 5 μg tRNA. The untreated probes and molecular weight markers were also loaded on the gels. The quantities of RNA used and methods were as described in Fig. 2 legend.

this corresponds to a 2.1-kb major *S* mRNA. This is shorter than the sizes of 2.3 or 2.45 kb for the HBsAg-associated transcripts estimated by Northern analysis<sup>1,2</sup> and correlates with the fact that we did not detect the two different start sites, assigned by indirect evidence, next to TATA-like sequences 500 or 650 nucleotides upstream of the *S* gene<sup>1,2</sup>. In contrast, we have demonstrated by nuclease digestions, primer extensions and promoter-specific *in vitro* transcription, that the major *S* gene transcript initiates 185 nucleotides upstream of the *S* gene. However, the sensitivity of our measurements does not exclude the existence of an additional, at least ten times weaker promoter upstream of the pre-*S* region.

The major *S* gene transcript we mapped encodes only 185 of the 489 nucleotides of the so-called pre-*S* region<sup>6</sup>, implying that pre-*S* is not part of an HBsAg precursor. Despite this fact, the transcript starting 185 base pairs (bp) upstream of the *S* gene contains a second AUG 15 nucleotides downstream of its start site, from which a minor HBsAg-related protein<sup>22</sup> of molecular weight 31,000 can be translated.

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Thus, we believe this to be the first study of the structure of HBV transcripts in infected liver. In this organ, as in an HBV DNA-containing cell line, HBsAg is translated predominantly, if not exclusively, from a transcript that is 2.1 kb long and unspliced. This transcript initiates close to the structural gene and terminates with variable efficiency more than 1 kb downstream of it. The *S* gene promoter shares extensive sequence homologies with two elements of the SV40 late promoter.

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After submission of this manuscript, Stenlund *et al.*<sup>23</sup>, using an extrachromosomal eukaryotic vector, mapped the same HBsAg mRNA 5' end that we identified, but they misinterpreted it as a splice acceptor site.

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## Dependence of Z-DNA antibody binding to polytene chromosomes on acid fixation and DNA torsional strain

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There is considerable interest in the existence and significance of alternative conformations of DNA<sup>1–4</sup> to the right-handed B-form described originally by Watson and Crick<sup>5</sup>. The indirect immunofluorescence observations of Nordheim *et al.*<sup>6</sup>, Arndt-Jovin *et al.*<sup>7</sup> and Lemeunier *et al.*<sup>8</sup> that antibodies against left-handed Z-DNA bind to polytene chromosomes have thus assumed considerable importance. However, there is a paradox: some workers observe Z-DNA in interbands and others in bands. We report here that binding of Z-DNA antibodies to *Drosophila* polytene chromosomes prepared without acid fixation is at background level, and that following acid fixation the same antibody treatment leads to intense fluorescence. Depending on the extent of exposure to 45% acetic acid, fluorescence can occur primarily in interbands or in bands. Furthermore, antibody binding is dependent on elastic torsional strain in the DNA molecules.

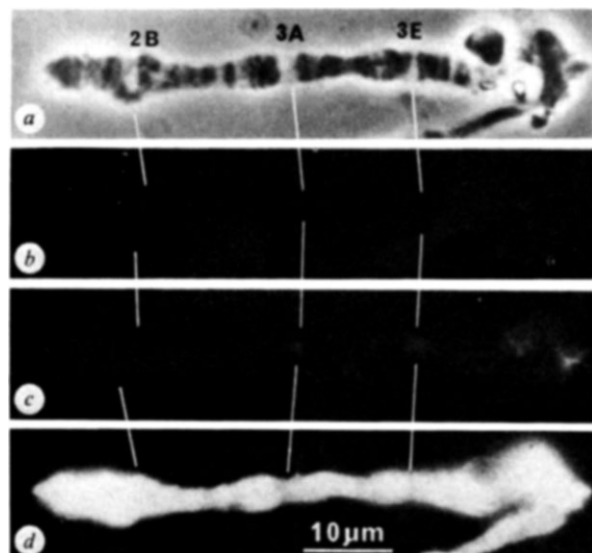
Since the 1930s the classical procedure for the cytological preparation of *Drosophila* salivary chromosomes has been carried out in the fixative 45% acetic acid<sup>9</sup>. Over the past years one of our groups has been developing a delicate microsurgical procedure for the isolation of salivary chromosomes at neutral pH and physiological ionic strength<sup>10</sup>. We refer to such chromosomes operationally as 'native', in the sense that they have not been exposed to denaturing solvents, only to conditions which should maintain higher orders of macromolecular structure. We

have found a short period of time toward the end of the third instar during which the chromosomes detach from the internal surface of the nuclear membrane. Taking advantage of this, we have been able to operate on the nucleus and withdraw the entire chromosome complement with fine glass needles. The chromosomes are released in D'Angelo's buffer<sup>11</sup> at pH 7 and physiological ionic strength. The mechanical strength of these fragile filaments can be increased by exposure to approximately two orders of magnitude lower concentrations of formaldehyde than that commonly used in variations of the classical procedure to combat the extraction effects of 45% acetic acid<sup>12</sup>. Alternatively the chromosomes may be released into buffer A of Hewish and Burgoyne, in which they are stabilized only by physiological concentrations of spermine and spermidine<sup>13</sup>.

The preservation of ultrastructure and nucleosomal organization of chromosomes isolated in this way is better than that of classical preparations<sup>14,15</sup>. Figure 1a depicts the distal region of the X chromosome isolated by this procedure; it has been slightly stretched to optimally resolve bands and interbands. On treatment with rabbit antiserum against Z-DNA followed by fluorescein-conjugated goat anti-rabbit IgG, the level of fluorescence was negligible—the same as that given by pre-immune rabbit serum (Fig. 1b). However, if the chromosomes were exposed to 45% acetic acid for 5 s, returned to neutral pH, and retreated with antibody, distinct fluorescence was apparent. This was concentrated predominantly in interbands and puffs (Fig. 1c). After 25 s exposure of the chromosome to 5% acetic acid and antibody treatment there was a massive increase in antibody binding and, furthermore, it was now predominantly over bands.

This progression of binding, first to interbands and then bands with increasing exposure to acid, is a reproducible phenomenon exhibited by both the X chromosome and autosomes. Binding to bands was observed with either whole serum or with immunospecifically purified antibody that was prepared from precipitates of serum and poly(dG-dC)·poly(dG-dC) in 4 M





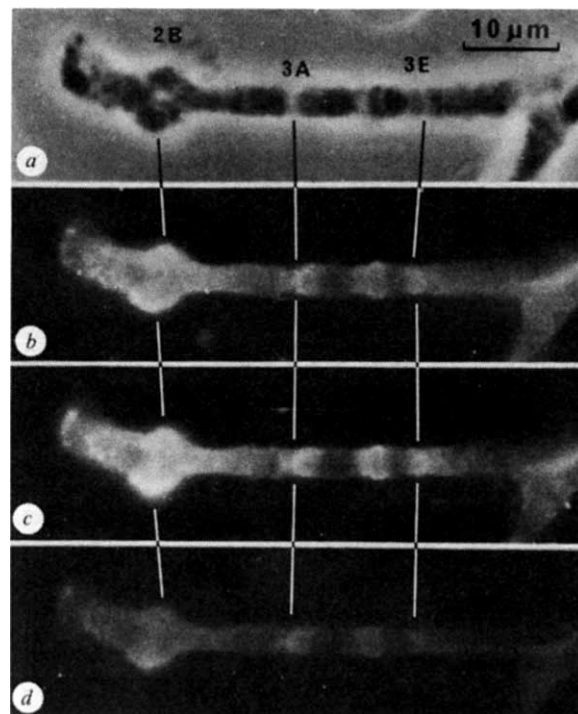
**Fig. 1** Phase contrast (a) and Z-DNA antibody-stained fluorescence micrographs (b–d) of the distal region of the X polytene chromosome. The chromosome was isolated by the procedure of Hill and Watt<sup>8</sup> without exposure to 45% acetic acid. In this procedure the chromosome was liberated into the phosphate-buffered saline of D'Angelo<sup>9</sup> containing 0.05% formaldehyde. In b, it was treated for 40 min with 1:50 diluted Z-DNA antiserum in D'Angelo's saline, washed and subsequently treated for 40 min with 1:20 diluted Miles fluorescein-conjugated goat anti-rabbit IgG. In c, it was treated with antibody after 5 s exposure to 45% acetic acid and return to phosphate-buffered saline at pH 7.0. Fluorescence is predominantly in interbands and puffs. In d, it was antibody treated after 30 s exposure to acid. Intense fluorescence now extends over the entire chromosome. Scale bar, 10 µm.

NaCl. Thus the additional binding to bands, not seen in the earlier work<sup>6</sup>, is due to antibodies that bind to Z-DNA, and not some other serum component. A similar acetic-acid-induced enhancement of binding of Z-DNA antibodies, from background to intense fluorescence, was observed with chromosomes isolated by microdissection in buffer A of Hewish and Burgoyne.

Could the lack of antibody binding to the native chromosomes simply result from inaccessibility of the DNA? This question was addressed by examining the binding of antibodies reacting with the B-form of DNA. These antibodies bound immediately to the native chromosomes (Fig. 2b). Clearly, the DNA is available for antibody binding. Furthermore, on treatment of the chromosomes with acid, there was no significant enhancement of B-DNA antibody binding and no shift in the pattern of binding (Fig. 2c, d). It is particularly clear that in loci such as puff 2B, antibodies reacting with B-DNA are binding to their antigen in the native chromosome (Fig. 2b) whilst antibodies against Z-DNA do not bind before acid fixation (Fig. 1b). Incidentally, the absence of Z-DNA antibody binding to what must be accessible B-DNA in such structures gives an additional criterion of the specificity of the Z-DNA antiserum.

What is the mechanism whereby the Z-DNA antibody binding is enhanced by exposure of the chromosomes to 45% acetic acid? The stringent tests of Lafer *et al.*<sup>16</sup> and Nordheim *et al.*<sup>6</sup> argue very strongly for specificity of our Z-DNA antiserum. Acid treatment could simply be unmasking Z-DNA present in the native chromosome. Specific Z-DNA binding proteins have recently been observed by Nordheim *et al.*<sup>17</sup> and perhaps these render the Z-DNA in native chromosomes completely inaccessible to antibodies. However, in view of the accessibility of the DNA to B-DNA antibodies, the general and massive activation of Z-DNA antibody-binding after exposure to acid, and the qualitative variation in the nature of the pattern of antibody binding, a second explanation is worth considering.

Exposure to 45% acetic acid can extract the four core histones of nucleosomes from chromatin and this extraction is detectable

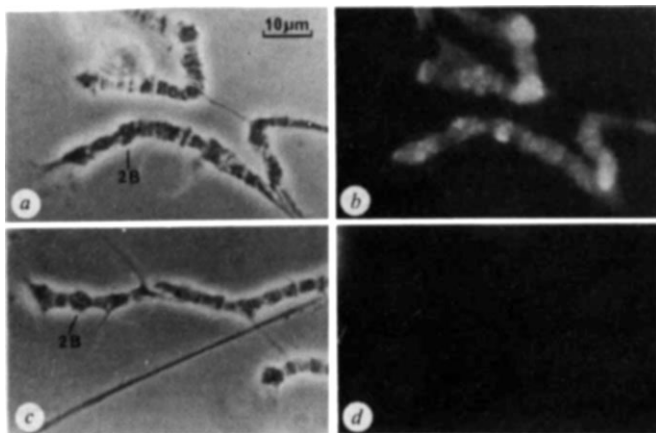


**Fig. 2** Phase-contrast (a) and B-DNA antibody-stained fluorescence micrographs (b–d) of the distal region of the X chromosome isolated by microdissection. The tip of this chromosome is in a slightly different focal plane and is consequently less well resolved. In b, the chromosome was treated with mouse monoclonal antibodies that react with B-DNA for 40 min, washed and subsequently treated for 40 min with 1:20 diluted Wellcome fluorescein-conjugated rabbit anti-mouse IgG. The characterization of the monoclonal B-DNA antibody will be described elsewhere. In c, it was treated with antibody after 5 s exposure to 45% acetic acid and return to phosphate-buffered saline. In d, it was antibody treated after 30 s exposure to acid. There is no significant increase in fluorescence after acid treatment (if anything there may be a slight decrease at 30 s).

even after formaldehyde fixation (ref. 18 and our own unpublished results). The removal of core histones from deoxyribonucleoprotein releases negative superhelical turns into the DNA<sup>19</sup>. The associated energy of deformation could drive the transition from the B- to the Z-conformation<sup>20–22</sup>. If this mechanism were responsible for the acid-mediated activation of Z-DNA antibody binding, then relaxation of supercoiling should destroy the antibody binding potential.

The effect of topoisomerase I on antibody binding is shown in Fig. 3. Figure 3a, b depicts the level of Z-DNA antibody binding to a chromosome that has been exposed to topoisomerase I and then to 45% acetic acid for 30 s. Figure 3c, d shows a similar experiment, except that the chromosome was treated with 45% acetic acid and then topoisomerase I before Z-DNA antiserum treatment. Topoisomerase I, after but not before the acid treatment, lowers the antibody binding to background. Similar experiments using a nicking concentration of DNase I (5 ng ml<sup>-1</sup>) after exposure of chromosomes to acid, also lowered Z-DNA antibody binding to background. This DNase I treatment did not lower B-DNA antibody binding (not shown), indicating that the enzyme was neither removing DNA nor blocking it. Thus the Z-conformation recognized in these chromosomes after exposure to acid is dependent on torsional strain in the DNA molecules. Both the Z-conformation and the supercoiling associated with it, are only detectable after exposure to acid.

A negative superhelical density in the range of 0.03–0.07 is sufficient to drive alternating dG-dC sequences of at least 14 base pairs (bp) in length towards the Z-conformation<sup>20–22</sup>. The superhelical density that can be induced by disruption of nucleosomes would be expected to be approximately 0.05 (calculated



**Fig. 3** The effect of topoisomerase I on Z-DNA antibody-binding. *a*, Phase contrast, and *b*, Z-DNA antibody-treated X chromosome that had been exposed to topoisomerase I (0.2 U  $\mu\text{l}^{-1}$  calf thymus enzyme for 30 min at 37 °C) and then 45% acetic acid (30 s). *c*, Phase contrast, and *d*, antibody-treated X chromosome that had been exposed to 45% acetic acid (30 s) followed by topoisomerase I. In *b*, the level of fluorescence is typical of an acid-treated chromosome. It is reduced to background in *d*.

from closed circular DNA extracted from SV40 chromatin<sup>19</sup>. It is of interest that Hamada *et al.*<sup>23</sup> have reported the equivalent of  $2 \times 10^3$  copies of alternating dT-dG sequences, 50 bp long, with Z-DNA forming potential in the *Drosophila* genome. It has recently been shown<sup>24,25</sup> that such (dT-dG)-sequences can be driven from the B- to the Z-conformation by a superhelical density of 0.047.

It is quite possible that 45% acetic acid (apparent pH 1.6) may facilitate the transition from B to Z by a number of mechanisms in addition to providing torsional free energy. (1) In Z-DNA, the base pairs are rotated 180° away from the position they have in B-DNA<sup>1</sup>. Strand separation at lower pH may well facilitate this rotation. (2) In Z-DNA, guanosine is in the *syn* conformation<sup>1</sup>. There is optical evidence consistent with a change from the *anti* to the *syn* conformation for guanosine on protonation of the base<sup>26</sup>. (3) Protonation of the primary phosphates in DNA, which has begun by pH 1.6 (ref. 27), might favour the Z-conformation in which phosphate groups are closer than in the B<sup>1</sup>. (4) The incorporation of DNA into nucleosome core particles stabilizes the B-conformation relative to the Z<sup>28</sup>; conversely, release from a nucleosomal organization should intrinsically favour a B to Z transition.

In conclusion, we have demonstrated that: (1) Z-DNA antibodies display only background-level binding to native *Drosophila* polytene chromosomes; (2) the binding is massively enhanced by prior exposure to 45% acetic acid; (3) depending on the extent of acid exposure, binding can be localized primarily to interbands or bands; and (4) the binding is dependent on torsional strain of supercoiling that, like the binding itself, can only be detected subsequent to acid treatment. This is the first demonstration that the Z-conformation in a cytological preparation of eukaryotic chromosomes is dependent on torsional strain. We have also shown that antibodies reacting with B-DNA have access to DNA in the native chromosomes.

These findings indicate that a significant proportion of DNA, in both bands and interbands, at least has a propensity to adopt a Z-conformation. However, the possibility that DNA, in chromatin, may be driven towards the Z-conformation by the liberation of torsional stress on the disruption of nucleosomes is a very real one, especially at low pH in acid fixatives. The fact that the torsional stress that may be generated on core histone extraction is in the range that can drive B to Z transitions, necessitates great care in extrapolating to *in vivo* conformations from the properties of chromosomes prepared in 45% acetic acid. Furthermore, the demonstration, described above, that variation in the conditions of acid fixation can lead to qualitative variation in the pattern of anti-Z antibody binding,

offers a likely explanation for the paradox presented by different interband and band binding patterns observed in different laboratories to date<sup>6-8</sup>. We have also recently observed that exposure of native chromosomes to 95% ethanol also leads to Z-DNA antigenicity, although to a lesser extent than 45% acetic acid (unpublished data).

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## Common site of integration of HTLV in cells of three patients with mature T-cell leukaemia-lymphoma: a retraction

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WE recently reported in this journal (Hahn *et al.* **303**, 253-256; 1983) that DNA from one fresh cell sample and two cell lines that are positive for HTLV contained provirus integration at the same site while 10 other HTLV-positive DNA samples did not. The conclusion was based on finding a rearranged DNA band that hybridized to a unique flanking cellular sequence called S2. In a note added in proof, we stated that an additional patient contained HTLV at the same locus. We have since carried out an extensive survey of HTLV-positive cells and have not seen a recurrence of a rearranged S2 band. In re-evaluating the early data, we found that the DNA samples that contained rearranged S2 sequences were contaminated by CR1, a recombinant phage containing HTLV and S2 sequences. This was not found in any of the other HTLV provirus-positive fresh cells or cultured lines.

Therefore, although we have evidence for HTLV provirus in many fresh leukaemic cell samples and cell lines, Wong-Staal *et al.*, *Nature* **302**, 626-628; 1983; and unpublished data), so far there is no evidence for a common integration site. Furthermore, the cell line HUT78 is provirus-negative.



# Human strata

Edmund Leach

**The Identity of Man: As Seen by an Archaeologist.**

By Grahame Clark.

Methuen: 1983. Pp.224. £12.50, \$17.95.

LONG before 1952, when Grahame Clark became Disney Professor of Archaeology at Cambridge, he was exercising a powerful influence on his archaeological contemporaries. Subsequently he was for many years the acknowledged leader of his academic profession in the British Isles. This book was written during the year following his retirement from his final employment, a seven-year stint as Master of Peterhouse, Cambridge.

A first casual glance suggests that this is yet another version of the *Outline of World Prehistory* (Cambridge University Press), first published in 1961 and radically updated on several subsequent occasions. In fact, despite overlaps of substance and attitude, the new book is an altogether more personal affair; instead of a bird's eye view of global archaeology we have the reactions of a famous archaeologist to the experience of making a series of such global assessments. What then is Man?

Man makes Society; Society makes Man. The societies which Clark admires are those whose archaeological residues contain artefacts which stir his aesthetic sensibilities. He calls them "high cultures". They were hierarchically ordered. He is indifferent to the social injustices which are inseparable from such hierarchy. At the very end of his book he quotes with approval a remark made by Renoir in his old age: "Italian painting is the work of a few Borgias, Medicis and other tyrants whom God blessed with a taste for colour".

But before we get to high cultures and hierarchy we have chapters about hominid evolution, the "genesis of cultural diversity" and "the findings of ethnography". These are very large subjects. The interest here is not in what they are but in how Clark perceives them to be.

War service apart, Grahame Clark has been associated with the Cambridge faculty ever since his undergraduate days. When he took his first degree in 1929 the academic fields of prehistoric archaeology, social anthropology and physical anthro-

pology, which are now taught in three distinct University departments, were not only the responsibility of a single faculty board, as is still the case, but were all fully integrated within a single tripos examination. Without nostalgia Clark sets out to remind the social anthropologists, the biological anthropologists and the prehistoric archaeologists of the present day that their respective specialities are still of mutual interest.



I have personal sympathy for that objective though I believe that the intellectual exchange that is called for is of a different kind from that suggested in this book. In so far as Clark is calling for a reintegration of prehistoric archaeology and social anthropology it is clearly the functionalist, "everything fits together like the gear wheels of a watch", kind of social anthropology advocated by Malinowski which he has in mind (p.67; Fig. 17). This is not what contemporary social anthropologists think they are talking about.

Prior to about 1910 it was generally assumed that when ethnographers engaged

in field research among "primitive" peoples, the societies and customs about which they wrote could be regarded as anachronistic survivals from remote antiquity. Those who studied such oddities thought of their subject matter as living fossils. There was therefore an immediate shared interest with the physical anthropologists who studied actual, but very dead, fossils, and with the prehistoric archaeologists who studied the material residues of actual, but very ancient, human societies.

By 1929 this way of looking at things was itself coming to seem like a survival from remote antiquity, though the Cambridge department had not kept up with changing fashions. Elsewhere the social anthropologists were insisting that the people they studied belonged to the present day. The variety of human culture was interesting in

its own right rather than because it might represent stages in the evolution of human society which had culminated in the triumphs of nineteenth-century Western capitalism.

The social anthropologists thus found themselves participating in a dialogue with conventional historians and with the sociologists who held similar views about the significance of cultural diversity, rather than with the prehistoric archaeologists whose commitment to the excavation of stratified sedimentary deposits gave them an *a priori* bias towards a stage-by-stage, evolutionary interpretation of their data.

On the social anthropological side of this divide, it is now only in the Communist countries that, for strictly doctrinaire Marxist reasons, we still encounter a consistently evolutionist interpretation of the variety of ethnographic fact. By contrast, Western archaeologists are still prepared to transport the facts of present-day ethnography into the remote past in order to flesh out the bare skeletons of ancient society that are provided by the immensely ingenious techniques of modern excavation. In the present context this is an odd conjunction of attitudes since Grahame Clark's personal political convictions lie well to the right of those of Margaret Thatcher.

Yet on this issue Clark is quite explicit. Although he rejects the simplistic stage-by-stage, unilinear social evolution postulated by Morgan and Marx, he insists that "the archaeological record documents in overwhelming abundance a cumulative advance in technology" (p.61). Cultural

variety may be interesting in itself but in the end it is the men who have the resources to subsidize the arts who really matter; at this level the process of humanization runs parallel to the progress of technological sophistication, and to the political domination of rulers over ruled.

The converse of this line of argument is the belief that the records concerning "indigenous peoples" as observed "at the time of initial contact with western man" link up directly with the findings of archaeology which "are capable of providing a tangible record of the process of humanisation from the first steps taken by the earliest Primates recognizable as men" (pp.86-87). Apparently we are expected to assume that, until "western man" came along, "indigenous peoples" everywhere pursued a "traditional" pattern of culture which had been virtually unchanged for centuries, perhaps even for millennia. Hence studies of Australian Aborigines or of Greenland Eskimo or of Kalahari Bushmen, when appropriately edited to remove the consequences of recent Western influences, can be used as evidence of the way the earliest members of our species solved the problems of adaptation to a variety of environments.

In fact there is no evidence that, in general, prehistoric societies were more stable than historic societies. Clark is aware that societies of widely different types may operate with very similar technologies and very similar material equipment and also that societies which are, from a sociological point of view, strikingly similar may be adapted to quite different environments and make use of quite different sets of material equipment; but he fudges the issues. He seems to want his readers to believe that the chaps with the better gadgets are "better" chaps in some overall human sense, either because technological sophistication implies better adaptation to the environment or because the making of objects for conspicuous display is a marker of social hierarchy, and the development of social hierarchy is what humanization is all about. But humanization is also all about cultural diversity; diversity for its own sake; social identification with small groups rather than large ones.

And that last qualification means that Clark stops well short of being an admirer of high technology in its modern, world-pervasive sense. On the contrary, everything about contemporary Western society which is contaminated with "egalitarian notions" and "the advance of homogenization" is a marker of *dehumanization*. Humanity reached its peak under the Ancien Régime; the rot set in with the French Revolution (p.151) and things have been getting worse ever since. Montesquieu who first taught his eighteenth-century contemporaries to admire cultural diversity would certainly have felt the same. □

*Sir Edmund Leach is a Fellow of King's College, Cambridge.*

## Holding the atomic reins

Robert Press

### Nuclear Power Struggles: Industrial Competition and Proliferation Control.

By William Walker and Mans Lönnroth.  
*George Allen & Unwin: 1983. Pp.204.  
£13.95, \$19.95.*

THE underlying issues addressed by the authors of *Nuclear Power Struggles* are twofold: a perceived weakening in international agreements intended to stem the proliferation of nuclear weapon development, and a struggle for survival among the world's leading nuclear manufacturers. The analyses and "crystal ball" views are set within the next ten years. This the reader may regard as a remarkably short period over which to assess a technology in which the introduction of even one new nuclear reactor could require as long, or even longer, and within which public attitudes towards nuclear power could change dramatically.

As background to their examination of the political, strategic and trading ramifications of changes in the nuclear industry over the past three decades, the authors have provided a useful, well-researched review of the industrial scene. They illustrate how growing competition in the nuclear power plant industry — particularly in the 1970s — led to US hegemony in industrial nuclear production, later giving way to a pluralism involving the US, Canada, France and Germany. And how, at the same time, US non-proliferation policies were inducing a rupture in international nuclear relations, threatening the very Non-Proliferation Treaty (NPT) which the US was seeking to reinforce. Here it seems strange, bearing in mind Britain's pioneering work (especially in the development of civil nuclear power) that the authors should attribute British resistance to US technology to "a Britain suffering from a post imperial hangover". Moreover, they virtually dismiss Britain as a participant in the evolution of nuclear development, including her international role stretching back to the 1950s in seeking nuclear arms control and anti-proliferation measures.

In dealing with non-proliferation initiatives during the same period, the authors are also less than fair to the International Nuclear Fuel Cycle Evaluation (INFCE) exercise, launched by President Carter in 1977 and open to all IAEA members. They suggest that dominance by the representatives of the nuclear community "prevented a realistic assessment of the prospects for nuclear power or for the Fast Breeder Reactor and reprocessing". They do, however, recognize INFCE's contribution in reducing the international

temperature in nuclear relations and in defining an agenda for further negotiations.

The subsequent analysis of the possible future course of the nuclear reactor industry is focused on market prospects and on political factors in the US, Canada, France and Germany (with a look ahead to Japan as the main contender to join those nations as a leader in the development of reactor technology). Hardly surprisingly, Britain is not included in this list. The book does, however, reveal that Britain and France are the only two countries who can offer a full nuclear fuel cycle to third countries; but, beyond noting that fact, Britain is again relegated to non-player status, notwithstanding her success in commercial re-processing.

The authors record that a twenty-year assumption about the efficacy of Light Water Reactors is now being questioned inside and outside the industry, and they therefore envisage a reassessment of nuclear technology policy. In this they notably "discount the Gas Cooled Reactor" — notwithstanding its well proven basic technology, its good safety characteristics and plans to have about 7,000 MWe of such capacity in operation in Britain by the time the first Pressurized Water Reactor could be commissioned. It would be interesting to see the British Central Electricity Generating Board's reactor load factors compared with those in the authors' relevant table of nuclear plant performance.

Turning to the question of non-proliferation, the authors consider that, at least for the time being, the generalized fear of weapon proliferation as an unavoidable by-product of civil nuclear expansion has died away. This they attribute to fewer investments in commercial enrichment and reprocessing; they do not develop the possibility that a further factor may be the spiralling expense of nuclear weapons systems for deterrent purposes and the increasing debate about the likelihood of actual use of nuclear weapons. Although they recognize the failure of policies of strict denial in trading nuclear materials (as developed in the 1970s, which exacerbated the mistrust created by nuclear suppliers in respect of Article IV of the NPT), the authors conclude that the best prospects lie in persisting with a policy of denial while making exceptions for reliable countries in reasonably stable regions. How one defines the latter exceptions is not clear. The increasing difficulty of applying general rules to nuclear trade is accepted, but the authors skate round a basic constitutional difficulty for the IAEA — the latter cannot discriminate between NPT and non-NPT parties. And while the authors find "some justification" for the non-nuclear-weapon states (NNWS), countries who argue against the NPT as discriminatory, they do not face the question as to whether some degree of discrimination is unavoidable when one starts from the simultaneous



existence of nuclear-weapon states (NWS) and NNWS. Much is made of NWS failure to implement Article VI of the NPT. But the extent to which this is hard reality or a cloak to preserve a weapon option is not considered.

*Nuclear Power Struggles* is confined within its subtitle. It does not enter on wider issues of nuclear energy for a long-term future; in this, one may perhaps be excused for sensing just a whiff of defeatism — something not evident in the attitude of the French. Yet within that restriction, and within the ten-year time-frame, the reader in government, industry or international organizations will find this a useful analysis of nuclear and industrial adjustment. On points of prediction, assessment or 'guesstimation' there is scope for challenge, but that does not minimize overall interest in the work presented. □

Robert Press was Deputy Secretary, responsible for Science and Technology, in the UK Cabinet Office from 1974 to 1976. He is now retired.

## Mineral matters

Roger G. Burns

### Orthosilicates. Vol. 1A of the second edition of *Rock-Forming Minerals*.

By W.A. Deer, R.A. Howie and J. Zussman.

Longman: 1982. Pp.912. £50, \$149.95.

THE publication of a new edition of a very successful, widely acclaimed reference text some 15–20 years after the previous edition always arouses interest and curiosity. Have the authors, now well into or beyond their prime, kept abreast of rapid developments in their field? Have they been able to cope with the voluminous literature published in the intervening years? Are new techniques, data and interpretations successfully assimilated into the text of the earlier edition? Is the revised edition as stimulating and as authoritative as the original? Fortunately, positive responses to these questions apply to two volumes that have appeared to date of the second edition of *Rock-Forming Minerals*, affectionately referred to by mineralogists as the DHZ volumes.

The original edition, including the five volume treatise *Rock-Forming Minerals* published in 1962 and the condensed version *An Introduction to the Rock-Forming Minerals* appearing in 1965 and widely used as an undergraduate textbook, provided a unique compendium of information about properties and occurrences of the most common minerals for over 20 years. Each volume in the first edition dealt with a specific group of structurally related minerals. They were the reference books to which researchers turned first for data on a mineral being

studied or for directions to an unsolved or poorly understood mineralogical problem.

However, the current generation of earth scientists, nurtured by the DHZ volumes, have themselves contributed to the proliferation of new information about minerals and rocks, rendering the first edition out-of-date. During the past two decades automated instrumental methods such as electron microprobe analysers and single crystal diffractometers have been developed, generating voluminous chemical and structural data for minerals; new spectroscopic and electron microscopic techniques have evolved, providing a wealth of new information about the crystal chemistries and microstructures of minerals, and wider ranges of accessible temperatures and pressures have extended experimental investigations of mineral syntheses and stabilities. In attempts to update the expanding data base for minerals, mineralogical societies have been conducting annual short courses and preparing inexpensive typewriter-offset printed volumes devoted to specific mineral groups, as shown by the *Reviews in Mineralogy* series published by the Mineralogical Society of America. It is against this proliferation of mineralogical data and the publication of competing texts that this second edition will be judged.

Of the two volumes published so far, each represents a bifurcation of an original volume. Vol. 2A, *Single-Chain Silicates* appeared in 1978 and covered minerals of the pyroxene and pyroxenoid groups. In Vol. 1A, *Orthosilicates*, minerals of the olivine and garnet groups are exhaustively covered, in addition to the humites, zircon, sphene, vesuvianite, sillimanite, mullite, andalusite, kyanite, topaz, staurolite, and chloritoid. The new editions retain the characteristics of the original volumes. Each mineral is discussed in five sections: structure, chemistry, optical and physical properties, distinguishing features, and paragenesis. Sometimes, additional sections on cation distribution and experimental work are added, reflecting the wealth of new data now available from spectroscopic measurements and mineral synthesis and stability studies. Numerous subheadings throughout the books also help clarify substantial new information. The text has been completely rewritten, and most of the illustrations are new and taken from recent literature. Tables of chemical analyses of each mineral have been expanded to include the plethora of electron microprobe results, and generally contain analytical data for only a few of the specimens described in the first edition.

The hallmark of the original volumes — comprehensive but not exhaustive bibliographies for each mineral — is reproduced in the second edition with references to publications as recent as early 1981. The treatment of orthosilicates in Vol. 1A compliments nicely the coverage in *Reviews in Mineralogy*, Vol. 5. The former contains more petrological information, is

better indexed, and more homogeneously written and printed than the latter, which abounds in crystal chemical and structural data for the minerals.

The excellence of the first volumes of the new edition heightens the anticipation of mineralogists for the publication of the rest of the series. They will remain the launching pad for the next generation of researchers of rock-forming minerals. □

Roger G. Burns is Professor of Mineralogy and Geochemistry at the Massachusetts Institute of Technology.

## Laws for the land

Richard Macrory

### Planning and Pollution: An examination of the Role of Land Use Planning in the Protection of Environmental Quality.

By Christopher Miller and Christopher Wood.

Oxford University Press: 1983. Pp.232.

£15.00, \$32.50.

ONE OF the more remarkable features of the British town and country planning system is the absence in the governing legislation of any clear statement of the purpose of planning controls. And this despite the fact that this field of law provides perhaps the country's most comprehensive intrusion of public control over private property rights.

It is possible to regard this lack of definition as a strength of the system, allowing it to adapt to particular political needs of the day. But one consequence has been an ever-present tension between planning professionals, the courts, Central Government and local planning authorities, as each attempt to redefine or push at the boundaries of current practice. Differing perceptions of the role of planning controls have raised fundamental questions: should planning be confined to questions of land-use conflict or 'amenity'; is it legitimate for planning authorities to investigate the economic viability or necessity of a proposed development; should the system be employed to further more overtly political or social goals, such as the discouragement of second homes, or the promotion of small businesses?

*Planning and Pollution* examines one area where there might be thought to be less room for conflict — the relationship between land-use planning and pollution controls. But although it was the growth of public health law in the nineteenth century that provided one of the mainstays for the development of the modern town and country planning system, pollution control laws now represent a distinctive body of specialized legislation. Responsibility for their enforcement often rests with agencies

quite separate from planning authorities, and, as likely as not, they will rest on regulatory principles dissimilar to those contained in planning laws.

The book is the distillation of a three year research study undertaken by the Pollution Research Unit of the University of Manchester. Essentially, the authors make a plea that, notwithstanding the existence of more specialized pollution laws, local authorities can, and should, use their planning powers to ensure that industrial developments take place with least environmental damage. As such, the book probably adds little more conceptually than was said on the subject in the 5th Report of the Royal Commission on Environmental Pollution in 1975. But where it gains is in the presentation of a number of recent case histories that illustrate the joint application of planning and pollution controls. In the main, they make for disturbing reading.

It is all too easy to criticize with the benefit of hindsight, but it is difficult not to feel concern at some of the examples given in the book. The planning decisions which permitted the juxtaposition of a housing estate and a waste disposal site, taken almost concurrently and with no apparent reference to each other; planning regulations which allowed a rug manufacturer to convert his premises for the manufacture of herbicides without the need for planning permission; the long history of conflict between a local planning authority and the Central Government Inspectorate responsible for air pollution control concerning a new sulphuric acid works, where the Inspectorate reported that, "We deprecate the insertion into planning consents of conditions which impinge on our statutory duties".

The authors conclude the study with a number of pertinent suggestions for improving the relationship and compatibility of the planning and pollution control systems. The current political climate, though, does not favour major legal or institutional changes in this field, as evidenced in the Government's belated and low-key response at the end of 1982 to the Royal Commission's 5th Report.

In the long run, the need to comply with the growing body of EEC policies concerning environmental protection, emphasizing prevention, may be seen to have wrought cohesive linkages between land-use planning and pollution control in the United Kingdom. For the present, greater appreciation of the difficulties that can occur through lack of coordination and misunderstanding between enforcement authorities may at least help avoid repetition of some of the worst instances recorded in *Planning and Pollution*. In this respect, the book is a study of value and importance. □

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## Photon reading

P. Mathis

### Light Reaction Path of Photosynthesis.

Edited by Francis K. Fong.

Springer-Verlag: 1982. Pp.342.

DM138, \$55.20.

WHEN a photon encounters a photosynthetic system it is likely to be absorbed by the so-called molecular 'antenna'; its energy then migrates to a reaction centre where charge separation takes place. When the reader approaches this book, like a photon, he immediately faces the complexity of a part of the antenna system, the phycobiliproteins. Fortunately, they are very nicely presented by H. Scheer who describes enough but not too much of the variability of these blue or red proteins found in lower oxygen-producing organisms. The photon-reader may then get a little lost in light-harvesting protein complexes covered by Cogdell and Beddard; the chapter is good overall, but the balance between details and generalities is uneven.

Before the reader reaches the reaction centre and is trapped by the primary electron donor he is prepared for the event by the good introduction to photosynthesis provided by A. Hoff. This chapter is an authoritative presentation of the primary partners in charge separation and of the spectroscopic tools used in their study. Hoff concludes by a more personal, and interesting, view on the bacterial reaction centre but leaves the reader a little confused (the photon has now irremediably disappeared): it would be interesting to know how the charges migrate away from the reaction centre, but this aspect is not covered in the book.

The chapters by Norris and Levanon on "Triplet state and chlorophylls" and by Clarke on "The chlorophyll triplet state and the structure of the chlorophyll aggregates" are both of the highest quality but they overlap considerably with each other and also with Hoff's chapter which results in a great deal of emphasis on triplet states.

A fascinating chapter, expertly written by M. Wasielewski, is devoted to synthetic models. This is an important and rapidly developing field: modelling the reaction centre helps our understanding of the biological reactions and brings closer the possibility of practical artificial photosynthesis. It is therefore unfortunate that the book has taken so long to be published: most of the references are to work published before 1979. The many printing mistakes in the text are also to be regretted.

The first and last chapters, both written by the editor F. Fong, express very personal views and it is perhaps unfair to insert the other chapters, which are extremely good and honest, between Fong's theories, implicitly presented as the state of the art in photosynthesis research. However, these

problems should not detract from the interest of the book which should be a useful addition to most research libraries. □

*P. Mathis is Associate-Head of the Biophysics Laboratory, Biology Department, C.E.N. Saclay, France.*

## Particle flows

K.G. Emeleus

### Electric Plasmas: Their Nature and Uses.

By A. von Engel.

Taylor and Francis: 1983. Pp.242.

£16, \$36.

THERE are comparatively few books which deal with both the properties and applications of gaseous plasmas and this one, by Dr von Engel, with many original contributions and comments is a welcome addition. The plasmas considered range from microdischarges to those in the earth's and sun's atmospheres, and in lightning and thermonuclear reactions.

References are appended to each chapter, and at the end to subjects axed — together with numerical examples — to keep the book of reasonable length. Even without what is left out of the main text, the field covered is extensive but the presentation is continuous and coherent.

The approach may be illustrated by Chapter 4, on the motion of charged particles in electric and magnetic fields. Except in very straightforward cases the theoretical and numerical analyses are approximations, but these do however usually yield results which come close to exact solutions. They are given first for pure fields, then under pre-plasma conditions, and finally for plasmas, and are discussed in detail. The corresponding subject sequence is; (no gas), boundary field refraction, electric and magnetic lenses, and oscillating fields; (pre-plasma), low and high field ion mobility, charge exchange, clusters, electron-drift, free and ambipolar diffusion, and self-repulsion; and (plasma), the Debye length, micro-fields, ranges of interaction, and the electrical and thermal conductivities of fully ionized gases.

On the applied side, there is again a wide range of topics, including amongst others triggering of gas explosions; shock tubes; lightning protection; arcs; corona discharges for spraying, copying and particle sorting; laser isotope separation; chemical changes; and flame radiation detection.

Altogether, Dr von Engel has given us an authoritative book, which is on the one hand an excellent introduction to the subject, and on the other, can be read by experts in the field with both enjoyment and profit. □

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## Awards

The Royal Society of Chemistry (RSC), UK, invites applications for the following awards: the RSC research fund, which is open to members of the society and is designed to assist them in current research; the Marlow medal and prize, awarded to members under the age of 32 for meritorious contributions to physical chemistry or chemical physics, determined on the basis of papers they have published, (the closing date for applications is 1 January 1984, on which date candidates must be under 34); the Sir Edward Frankland fellowship for the encouragement of research in the organometallic chemistry or the coordination chemistry of transition metals. The fellowship is awarded for a two-year period and the funds will be transferred directly to the fellow's institution. Candidates for the Sir Edward Frankland fellowship should be under 36 on 31 December 1983, which is the closing date for applications. The RSC Corday-Morgan memorial fund enables members of any established chemical society or institute in the Commonwealth to visit chemical establishments in any other Commonwealth country. The maximum award is £500, and the annual closing dates are 1 April and 1 October. In addition there are a Corday-Morgan medal and prize. Three prizes will be awarded for 1982, each consisting of a silver medal and prize money of 250 guineas. They will be given to British chemists under the age of 37 who have between them made the most worthy contributions to their fields of chemistry. The Sir George Beilby memorial fund, which consists of a medal and prize, is made to British investigators in recognition of work which has led to advances of practical significance. The RSC also offer the Meldola medal and prize, worth £100, which are given to the chemist who is under 30 years of age on 31 December 1983 and who shows the most promise as indicated by his published work. This must be brought to the notice of the RSC before 31 December 1983. The Carbohydrate Chemistry award, sponsored by Tate and Lyle, will be given to the member of the RSC who has published the most meritorious contribution to the knowledge of any aspect of carbohydrate chemistry, and who is under the age of 36. Finally, the society runs a scheme of awards to enable its members to visit chemical laboratories in developing countries.

Applications are invited for the 1984 Harkness Fellowships. Twenty fellowships will be awarded for study and travel in the USA. The fellowships are tenable for between 12 and 21 months and include return fares to the United States, living and family

allowances, travel in the US (with car rental allowance), tuition and research expenses, a book and equipment allowance and health insurance. Candidacy is open to those who have completed a minimum of four years' professional schooling as well as the major part of their further education (or equivalent professional experience) and who have not spent more than six months in the US between their nineteenth birthday and 1 September 1984. Further details of the application procedure are available from The Harkness Fellowships, Harkness House, 38 Upper Brook Street, London W1Y 1PE, UK, although forms will not be posted after 6 October.

Applications are invited for the Smithsonian Institution's programme of research training in higher education for 1984 in the fields of American history and material culture, history of art, history of science and technology, earth sciences, anthropology, materials analysis, and biological sciences. Smithsonian fellowships are awarded to support independent research, in residence at the Smithsonian Institution, related to the research interests of the Institution's professional staff and using the Institution's collections, facilities, and laboratories. Six- to twelve-month pre- and postdoctoral fellowship appointments and ten-week graduate student appointments are awarded. Applications are due on 15 January 1984. Stipends supporting the awards are \$18,000 per year plus allowances for postdoctoral fellows; \$11,000 per year plus allowances for predoctoral fellows; and \$2,000 for graduate students for the ten-week period of appointment. Pre- and postdoctoral stipends and allowances are prorated on a monthly basis for periods of less than one year. Application forms are available from the Office of Fellowships and Grants, 3300 L'Enfant Plaza, Smithsonian Institution, Washington, DC 20560, USA. Candidates are asked to indicate their proposed area of research.

Applications are invited for the second Sandoz prize for gerontological research. The prize, worth 20,000 Swiss francs, is designed to encourage research in all areas of gerontology and geriatric medicine, including biological, medical, psychological and social aspects, with special emphasis on multidisciplinary research programmes. Applications should be submitted in English before 15 September 1984 and should consist of a summary of the research work of between three and five pages. A curriculum vitae, bibliography and reprints of not more than three pivotal publications should accompany the application. The prize will be awarded during the International Congress of Geron-

tology, 12–15 July 1985.

The Wolfson Foundation has set up an industrial research fellowship scheme in the UK whereby industrially-oriented scientists and engineers who are out of a job may gain positions in any laboratory where industrially-oriented work is carried out. Applicants must propose a project on which to work, and stipends will be awarded according to the age and experience of the applicant and the laboratory of their choice. Preference will be given to applicants between 25 and 35, and only those with experience of industrial research and development will be considered. The scheme is being administered by the Fellowship of Engineering. Further details may be obtained from: The Fellowship of Engineering, 2 Little Smith Street, Westminster, London SW1P 3DL, UK.

The James Watt International Gold Medal (1983), presented by the British Institution of Mechanical Engineers, is being made to **Sir Christopher Cockerell** for his invention and subsequent development of the hovercraft. The IMechE makes this award biennially to an engineer of any nationality who has achieved international recognition both through his work as a mechanical engineer and through the ability with which he has applied science to the progress of mechanical engineering.

The Biochemical Society has made the following awards: the Colworth medal to **Professor Eric Oldfield** of the University of Illinois; the Wellcome Trust award to **Professor Robert Williamson** of St Mary's Hospital Medical School, London, UK; the Ciba medal to **Dr George Rada** of the department of biochemistry at the University of Oxford, UK; **Dr Edwin Krebs** of the University of Washington, USA is the 1983 Hopkins memorial lecturer, and **Dr Joseph Chayen** of the Kennedy Institute of Rheumatology, London, UK has received the BDH medal.

The Gregory Pincus medal and award have been given to **Patrick Steptoe** and **Robert Edwards** of the University of Cambridge, UK, who pioneered the first 'test-tube baby', Louise Brown. Dr Edwards and Dr Steptoe will give the 1983 Gregory Pincus memorial lecture on "Techniques of Human *in vitro* Fertilization and Embryo Replacement" at the Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts, USA on 17 October 1983. Further information on the lecture may be obtained from: Chairman, Gregory Pincus Memorial Lecture Committee, Worcester Foundation for Experimental Biology, 222 Maple Avenue, Shrewsbury, MA 01545, USA.

## Miscellaneous

The International Commission on Zoological Nomenclature (ICZN) is seeking contact with international representative organizations in all fields of zoology. A contact of the kind envisaged by the ICZN has already been set up with the International Palaeontological Association, whereby the organizations exchange information on cases of interest to both parties. Any organization that wishes to improve communications in matters of zoological nomenclature is therefore encouraged to approach the ICZN directly by contacting Richard Melville, ICZN, c/o British Museum (Natural History), Cromwell Road, London SW7 5BD, UK.

The Howard Heinz Endowment has awarded the University of Pittsburgh, USA, a five-year, \$2 million grant to expand the Heinz Nutrition Center. The centre was originally established in 1977 through a \$1-million grant from the Heinz Endowment through the Nutrition Foundation of New York City. Steps being taken in the expansion of the centre include the appointment of a director and the restructuring of the core laboratory for nutritional evaluations in the University Health Center of Pittsburgh.

Applications are invited for membership of the Society for Marine Mammalogy, a professional organization whose purpose is to help improve both marine mammal science and marine mammal management. The society was formally set up in December 1982 and membership is open to anyone who has produced a significant contribution to marine mammal science, or who has contributed substantially to the management, husbandry or training of marine mammals. The society will also welcome as associates all those who have an interest in pursuing its aims. Further information is available from Dr William Perrin, National Marine Fisheries Service, Fishery-Oceanography Center, PO Box 271, La Jolla, California 920378, USA.

The British Agricultural Research Council (ARC) has set up a new food division in response to recommendations in the ACARD (Advisory Council for Applied Research and Development) report on the food industry and technology which was produced in September 1982. The new division will be headed by **Professor Frank Curtis** for the first year. Professor Curtis will also retain his responsibility as director of the ARC Food Research Institute.

As part of a programme to encourage membership, the Chromatography Discussion Group has reduced the subscription rates of members under the age of 25 from £18.50 to £6.00 with effect from 1 January 1984. Further information can be obtained from Dr Charles Cook, Press Officer,

Chromatography Discussion Group, Trent Polytechnic, Burton Street, Nottingham NG1 4BU, UK.

The Royal Society of Great Britain and the trustees of Sir Henry Wellcome have announced that the scientific papers and correspondence of Sir Henry Hallett Dale (1875–1968) have now been prepared for preservation in the archives of the Royal Society, of which he was secretary from 1925 to 1935, and president from 1940 to 1945. The papers are open for consultation with the permission of the society's officers and a hand-list of them is available. The papers concerning Sir Henry Dale's chairmanship of the Wellcome Trust (1938–60) have been retained by the trustees.

## Meetings

2 October, **International Symposium of the Fulton Society on Brain Metabolism** to be held in conjunction with the **Annual Meeting of the American Neurological Association**, New Orleans (Professor Victor Soriano, Calle Buenos Aires 363, Montevideo, Uruguay).

11–15 October, **Oceanexpo and Oceanotropiques 83**, Bordeaux, France (Technoexpo SA, 8 rue de la Michodière, 75002 Paris, France).

18–22 October, **European Toxicology Forum**, Geneva (Francine de Greef, The Toxicology Forum, 14 avenue de l'astronomie (Box 29), B-1030 Brussels, Belgium).

20 October, **Annual Lord Downing Fund Lecture — Approaches to Toxicology and Experimental Medicine Using the Chick Embryo; an Alternative to the Draize Test**, London (The Lord Downing Fund, 51 Harley Street, London W1N 1DD, UK).

20–21 October, **Conference on Toxicity Testing**, London (Beverly Humphrey, Scientific Symposia Ltd, 33–35 Bowling Green Lane, London EC1R 0DA, UK).

24–26 November, **International Symposium on Vitamins in Nutrition and Therapy, with Focus on Vitamin C**, Cartagena, Colombia (Dr Camilo Rozo, Symposium Coordinator, Productos Roche SA, Apartado Aéreo 14437, Bogotá DE Colombia).

28 November–17 December, **International Training Course in the Physiology and Genetics of Viruses**, Dakar, Senegal (Professor B. Hirt, Institut Suisse de Recherches Expérimentales sur le Cancer, Chemin des Boveresses, CH-1066 Epalinges s/Lausanne, Switzerland).

23–24 November, **Mammalian Biochemical Genetics Workshop**, London (Dr G. Bulfield, Genetics Group, ARC Poultry Research Centre, Roslin, Midlothian EH25 9PS, UK).

10–11 November, **Conference on Translating and the Computer 5: Tools for the Trade**, London (Conferences Organizer, Aslib, 3 Belgrave Square, London SW1X 8PL, UK).

20–25 November, **Meeting of the World Congress, Alternatives and Environment**, Tel Aviv (The Secretariat, 2nd Meeting of the World Congress, Alternatives and Environment, PO Box 50006, Tel Aviv 61500, Israel).

5–10 December, **Meeting of the American Geophysical Union**, San Francisco (American Geophysical Union, 2,000 Florida Avenue, NW, Washington DC 20009, USA).

8–9 December, **Symposium on the Molecular Basis of Viscoelasticity**, Cambridge, UK (Mrs Y.A. Fish, The Royal Society of Chemistry, Burlington House, London W1V 0BN, UK).

12–21 December, **International Congress of Genetics**, New Delhi (Professor V.L. Chopra, Organizing Secretary, Division of Genetics, Indian Agricultural Research Institute, PO Box 2841, New Delhi-110060, India).

14–16 December, **Solid State Physics Conference**, Oxford, UK (M.R. Wells, The Clarendon Laboratory, Parks Road, Oxford OX1 3PU, UK).

20–21 January, **International Symposium on Musculoskeletal Disorders**, Switzerland (Symposium Secretary, UCLA Medical Center, 10833 Le Conte, Room 76-139 CHS, Los Angeles, California 90024, USA).

23–28 January, **International Course in Flow Cytometry**, Paris (Dr C. Rosenfeld, INSERM — Unité 253, Groupe Hospitalier Paul Brousse, 16 bis, avenue P.V. Couturier, 94804 — Villejuif, France).

29 January–2 February, **Summit on Allergy, Immunology, Pulmonology and Ear, Nose and Throat**, Keystone, Colorado (Ms H. Cole, NJH/NAC, 3800 East Colfax Avenue, Denver, Colorado 80206, USA).

6–8 February, **Consensus Development Conference on the Use of Diagnostic Ultrasound Imaging in Pregnancy**, Bethesda, Maryland (Ms M.P. Richardson, Office of Research Reporting, NICHD, Building 31, Room 2A32, 9000 Rockville Pike, Bethesda, Maryland 20205, USA).

12–18 February, **Advancing Agricultural Production in Africa**, Arusha, Tanzania (General Secretary, Advancing Agricultural Production in Africa, Commonwealth Agricultural Bureaux, Farnham House, Farnham Royal, Slough SL2 3BN, UK).

9–13 January, **Chapman Conference on Natural Variations in Carbon Dioxide and the Carbon Cycle**, Tarpon Springs, Florida (E.T. Sundquist, US Geological Survey, 431 National Center, Reston, VA 22092, USA).

19–25 February, **International Biotechnology Symposium**, New Delhi (The Chairman, Programme and Publication Committee, VIIth International Biotechnology Symposium, Biochemical Engineering Research Centre IIT Delhi, Hauz Khas, New Delhi — 110016, India).